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EXTENDING THE VASE LIFE OF MIXED FLOWER BOUQUETS

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EXTENDING THE VASE LIFE OF MIXED FLOWER BOUQUETS

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ABSTRACT

The popularity of mixed flowers bouquets has increased in the cut flower industry. The longevity of mixed flowers bouquets is limited by the individual life of each flower in the bunch. Various factors have been studied for their effect on the individual life of each flower in mixed flowers bouquets.

The results indicate that the differences in vase life of each flower in mixed bouquets may come from the influence of their phenotype. In cut rose flowers, there were various contents of fructose, glucose, sucrose and myo-inositol in all cultivars. However, levels of sugars contents were not related to the long-lived cultivars.

For cut lily flowers, the contents of fructose, glucose and sucrose were measured in the primary and the secondary bud of 'Tiber' lilies. Differences in the contents of fructose, glucose and sucrose between the primary and secondary bud, did not relate to the longevity of individual bud life. However, this experiment found increases in sucrose contents in both positions before the time of opening. This increase in sucrose may relate to the role of the carbohydrate energy source for bud opening.

Bacteria found in this study seemed to be largely specific to different flower types. Also, species of bacteria found in the vase water of mixed flowers were less than in that of single varieties. Difference in species of bacteria may be due to differences between plants, and the conditions they exert in the vase water. In general bacterial numbers were high at the beginning of all the experiments, indicating a strong inoculum effect from stems, although there was no straightforward relationship between stem numbers and inoculum size, in all cases.

Experiments of single varieties were studied using 'Tiber' lily, 'Akito' rose and 'Valentino' rose. The results indicated that the addition of stems did not result in higher numbers of bacteria, but vase life was reduced, especially for the roses.

The effect of number of stems in mixed flowers was investigated in distilled water and liquid flower food. The result of the addition of stems was to reduce the

vase life of both ‘Tiber’ lily and ‘Akito’ rose. However, the effect of more stems of ‘Akito’ roses had a greater effect on vase life, fresh weight and water uptake than in ‘Tiber’ lilies. This indicated that ‘Akito’ roses had a greater response to high bacteria populations than lilies.

The role of chemical exudates on flowers was not clear in the present study as they only had an effect during the early part of the vase life. The vase lives of ‘Tiber’ lilies and ‘Akito’ roses in all treatments were not significantly different. Perhaps treatment effects caused by the presence of chemical exudates may have been ‘masked’ by the presence of micro-organisms.

The screening of 12 essential oils (West Indian bay, cinnamon leaf, clove bud, clove leaf, Chinese ginger, lemongrass, mandarin, rosemary, sage, spearmint, sweet fennel, and thyme) showed that thyme oil and lemongrass oil are effective against more bacterial species over other essential oils. The comparison of the minimal bactericide concentration (MBC) of thyme oil and lemongrass oil showed that the average MBC for thyme oil was lower than that of lemongrass. However, the thyme oil failed to extend the vase life of ‘Tiber’ lily and ‘Akito’ rose. Thyme oil was effective against bacteria for a very short time and could not extend vase life, especially that of the ‘Akito’ rose. Overall, thyme oil treatment appeared to reduce vase life.

The efficacy of nine weak organic acids was studied in vivo for their effect on six bacterial species. Bacterial samples were inoculated on trypticase soya agar (TSA) pH 4 and 7. At pH 7, the TSA was prepared by using distilled water. All six bacteria species grew well under these conditions. The results of weak organic acids studied at pH 7 showed that Trans-cinnamic acid was the only weak organic acid that could act against some bacteria. At pH 4, only L8 (*Bacillus spp*.) could grow, and Trans-cinnamic acid was effective against this bacteria. The initial result of testing Trans-cinnamic acid against bacteria offered some promise for it to be applied successfully to the vase water of cut flowers, in combination with buffer systems.

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NOTATION

%	percent
±	plus or minus
C°	degree celsius
µ	micro
$	dollar
Ag	silver
ANOVA	analysis of variance
CO_2	carbon dioxide
CRD	completely randomized design
cm	centimetre
cfu	colony forming units
DICA	dichloroisocyanuric acid
DW	dry weight
e.g.	for example
et al.	and other
EU	The European Union
f.w.	fresh weight
HPLC	high performance liquid chromatogramphy
HQS	hydroxyquinoline sulface
i.e.	that is
in vitro	outside a living organism
in vivo	inside a living organism
LSD	least significant difference
Ltd.	limited

MBC	minimum bactericidal concentration
mg	milligram
mL	millilitre
mm	millimetre
m^2	squire meter
ND	not detected
NO.	number
NS	nanoparticle
pH	relative proton concentration in a solution
ss.	sum of squire
SD	standard derivatives
TCA	tricarboxylic acid
UK	United kingdom
US	United state
v/v	volume by volume
w	weight

CHAPTER ONE

Literature review

1.1 Introduction

Ornamental plants such as cut flowers, foliage and potted plants are an important part of the agricultural industry. With their aesthetic characteristics, ornamental plants are used to decorate houses and for celebration. In addition, ornamental plants, especially cut flowers, are used as gifts for many different occasions.

The largest market for ornamental plants is in Europe and America. This incurs transportation from many countries around the world to central markets. Long term transportation and environmental conditions from the production area to market often influence the quality of ornamental plants, especially cut flowers. Postharvest technology has been studied to improve quality and extend flowers' vase life or the shelf life of ornamental plants. The postharvest vase life of cut flowers depends on several factors, such as the species or variety, environment conditions, water supply and uptake, endogenous carbohydrate supplies, and the presence of microorganisms.

Due to the consumption of ornamental plants being related to social trends and fashion, there are new varieties of cut flowers and many patterns of arrangement that use various different cut flowers. Not only a single variety flower but mixed flower bouquets are popular in supermarkets. Due to the aesthetics of various cut flowers in the same bunch, this satisfies consumer demand. Mixed flower bouquets are a type of flower arrangement which is increasingly popular in the cut flower industry, and consist of mixes from various flower types such as roses, lilies, carnations, gerberas, orchids, etc. Mixtures of roses and lilies are particularly popular.

For mixed flower bouquets, the end of their vase life is indicated by advanced signs of deterioration (e.g. wilting or fading). In mixed bouquets, various types of flowers are used, such as single flower types (rose, gerbera, tulip, etc.) and inflorescence-types (lily, gladiolus, carnation). The vase life of a mixed bouquet is effectively limited by the life of the shortest living variety. The vase life of a single flower or the longevity of an individual bud in the same inflorescence influences the

vase life assessment of the mixed flower bouquet. Although mixed flower bouquets are now becoming extremely popular, the lack information on the compatibility of certain varieties has been a problem.

Due to a difference in the stages of development and vase lives of each flower, this may have an effect on the flower quality of each flower. To the author's knowledge, there is no previous work that has studied the effects of the interaction between each flower in mixed bouquets which may influence their vase life. This research project, therefore, intends to study various factors affecting flower quality and vase life of mixed flowers bouquets. The knowledge gained from this project may present ideas for the cut flower industry.

1.2 Cut flowers

Today, cut flowers have an important role in the floral industry. Besides home decoration, cut flowers are used for cultural ceremonies and used as a symbol of social expression. In 2008, floriculture products within the worldwide trade were estimated at over US$ 14 billion. The largest markets are Germany, the United States, Britain, France, and the Netherlands. Almost 70% of all imported floriculture products were consumed by these five countries (Maree and van Wyk, 2010).

The European Union (EU) consumes more than 50% of the world's flowers. Consumers request a high quality of flowers. Supermarkets also pay particular attention to quality requirements. The purposes for purchasing by consumers are (1) as gifts and on special occasions; (2) for personal use. The purpose of purchasing flowers is around 50-60% as a gift. Around 15% is for funerals and the rest is for personal use. Consumers expect the quality of flower to be high – not only freshness but a long vase life is important. Moreover, consumers are not only interested in the shape, colour or fragrance of flowers but increasingly are concerned about environmental issues during the process of production (CBI market survey, 2007).

1.2.1 Cut flowers in the United Kingdom (UK)

The UK is the second largest import country for cut flowers and foliage in the European Union (EU). About 75% of the cut flowers and cut foliage sold in UK markets are imported. Consumers buy flowers as a gift (49%); home decoration (31%); ceremonies and funerals (15%); and for other reasons (5%). Cut flowers sold as single species bouquets represent 43% of sales, while 41% is mixed bouquets and 16% is in arrangements and decorations (Commonwealth Secretariat, 2001). See Table 1.1.

Table 1.1: The major floral import products to the UK market (from Commonwealth Secretariat, 2001)

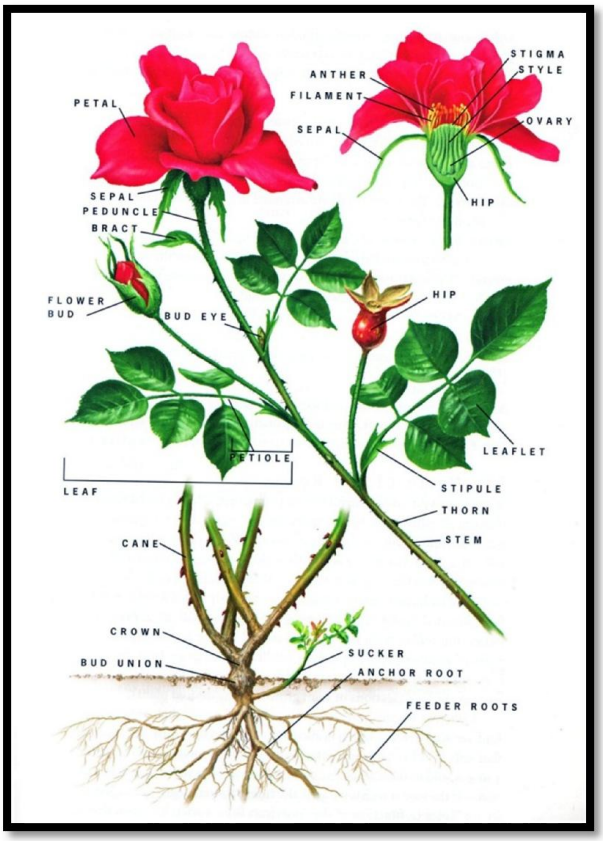
	1995		**1999**	
	EUROS	**TONNES**	**EUROS**	**TONNES**
Roses	26,851	4,547	53,477	8,365
Carnations	85,255	23,313	110,465	28,456
Chrysanthemums	46,515	11,572	84,482	22,912
Orchids	2,620	358	3,317	304
Gladioli	804	281	1,182	339
Others	103,399	23,750	236,162	43,320
Foliage	10,877	4,256	17,183	5,551

1.3 Trends of bouquet consumption

Consumer preference, patterns and purchasing patterns of cut flowers differ among countries in the EU. Affluent people buy more bouquets of exclusive flowers while less affluent people buy simple bunches of flowers. The emotional element is important for European consumers. Demand for bouquets is growing and a high labour input is required for the preparation of bouquets. Therefore, developing countries intend to export bouquets direct to supermarkets (CBI market survey, 2007). Consumers expect the vase life of a bouquet to be not less than seven days. The vase life of flower bouquets depends on various factors such as genetics, culture condition, harvest stage, postharvest conditions and the addition of 'flower food' (added ingredients including sucrose). The vase life of certain mixed flower bouquets can extend over seven days with suitable postharvest handling (Ranwala, 2007).

1.4 Roses

The rose (*Rosa hybrida*) is one of the most popular among cut flowers and is used mainly in bouquets and floral decorations. Roses are generally grown as cut flowers, potted plants, or plants in gardens. All plants in the genus *Rosa* are woody with thorns, spines, or prickly stems. The leaves are alternate and may be deciduous or persistent. The ovary develops into a fleshy fruit or 'hip' that changes from yellow to red when ripe. The prominent five part sepals (calyx) are leaf-like, covering the flower bud (Dole and Wilkins, 1999). Picture 1.1 shows the anatomy of the rose flower.



Picture 1.1: Anatomy of rose flower.

(Source:http://www.nankana.com/nankana_gardens/garden_resource_files/Roses/anatomy_of_a_rose.html)

There are 40-500 varieties of roses grown for commercial purposes. In the EU, one third of the total volume is imported. The major exporters are Kenya, Ecuador, Zimbabwe, Israel, Colombia and Zambia. The major importing countries are Germany, the Netherlands, France, Switzerland, the UK and Italy. Within the EU, the Netherlands is the main supplier of roses (Commonwealth Secretariat, 2001).

For hybrid tea roses, thousands of cultivars have been bred and divided into three categories; hybrid teas, sweethearts and spray roses. Hybrid teas are classified as large, intermediate and small. Vase life normally varies from 5-14 days (Maree and van Wyk, 2010). Picture 1.2 shows examples of rose cultivars that are grown for commercial purposes (and used in this project).

Picture 1.2: Examples of rose cultivars: 'Akito' (A), 'Blushing Akito' (B), 'Sweet Akito' (C), 'Tropical Amazon' (D), 'Red Calypso' (E), 'Valentino' (F), 'Viva' (G), 'Inka' (H) and 'Glossy' (I).

There are many factors that influence the longevity of cut roses such as cultivar, stage of development, optimized growth environment, production season, and postharvest handling (placing stems in warm water, using high quality water with an appropriate pH, using preservatives with sucrose, antimicrobials, ethylene inhibitors, air temperature, humidity, and light). Temperature should be controlled close to 0 to 1°C during handling. The optimal humidity should be controlled nearly 80% to reduce transpiration (Dole and Wilkins, 1999).

1.5 Lilies

At present, the lily is a popular flower for decoration. Over 200 million cut stems are sold in the UK each year. Lilies are from the genus *Lilium*, *Liliaceae* family (Jefferson-Brown, 2008).

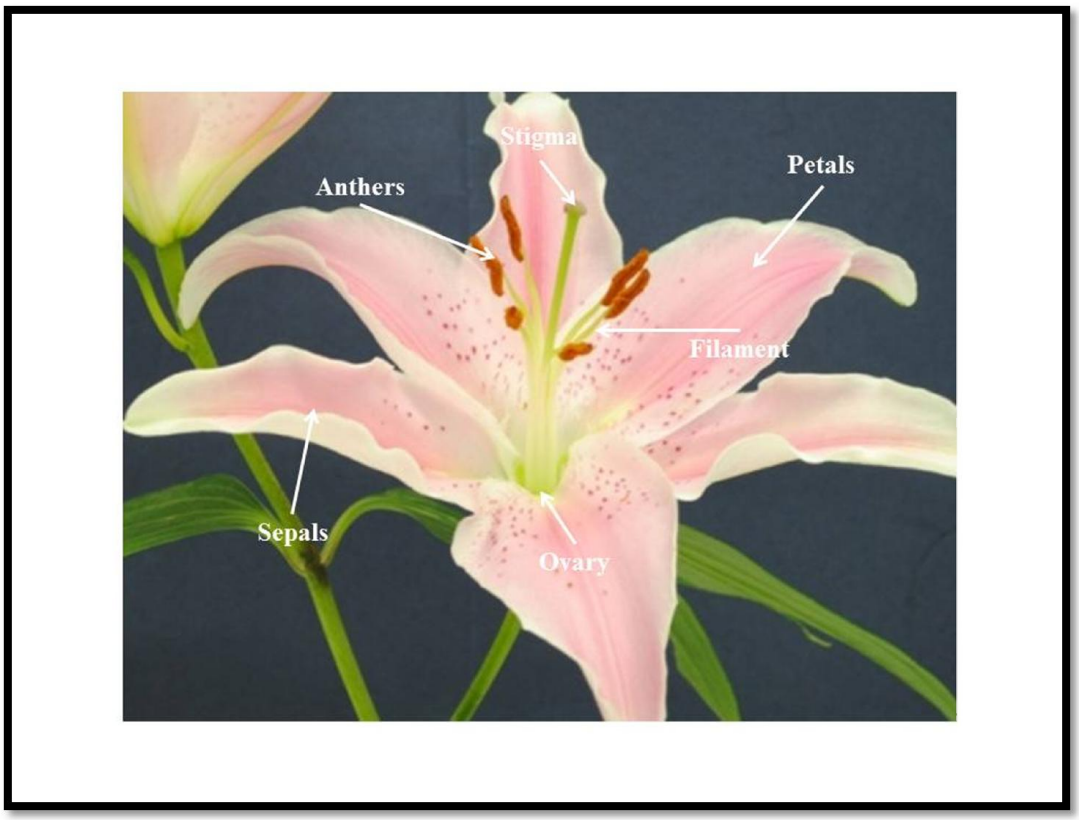
Hybrid lilies for the cut flower markets are divided into three groups, Asiatic, Oriental, and the hybrids between *Lilium longiflorum* and Asiatic lilies which are called 'L.A. hybrids' (Armitage and Laushman, 2003).

Oriental lilies are bulbous plants with inflorescences and outward-facing flowers. These lilies have a larger flower and are more trumpet-shaped than Asiatic lilies and also have more fragrance. Oriental cultivars were originally bred between *L. auratum* from Japan and *L. speciosum* from China and Japan. For cultivation, oriental lilies prefer a cool temperature (18-21°C) and high light intensity. Vase life varies from 5-9 days (Maree and van Wyk, 2010). Picture 1.3 shows the 'Tiber' lily and 'Mother's Choice' lily, which are grown for commercial purposes (and used in this project).

Picture 1.3: Oriental lily cultivar: 'Tiber' (A), 'Mother choice' (B).

Picture 1.4 shows the anatomy of lily flowers. All lily flowers consist of six 'petals', the outer three petals are really sepals. These three sepals may be more or

less identical to the three true petals, however the sepals may be broader. Botanically, petals and sepals are known as the perianth. Lilies have petals that enclose all sexual parts, with the ovary sited in front of the point of petal attachment. The ovary can produce over 100 seeds (Jefferson-Brown, 2008).



Picture 1.4: Lily anatomy.

The inflorescence of lilies can be a raceme, an umbel or a single terminating flower. A raceme is a series of flower stalk or pedicels together with the stem. The pedicel of the lily may either be branched or unbranched depending on the genetics and age of the plant. There are two or three or more buds on the branch. Generally, the buds always start to open with the primary bud, followed by the secondary bud until the final buds open. (http://www.mikesbackyardgarden.org/lilygen.html). The senescence of 50% of buds indicates the end of inflorescence longevity (Burchi *et al.,* 2005). Van der Meulen-Muisers *et al*., (2001) suggest that the vase life of lily flowers depends on the number of buds within the inflorescence after harvest. Picture 1.5 shows positions of buds in the inflorescence of a lily.

Picture 1.5: Position of the primary and secondary buds in the inflorescence of a lily.

1.6 Senescence of cut flowers

The vase life of flowers differs due to their species and variety. 'Vase life' is a variable used to indicate cut flower longevity (Joyce and Faragher, 2012). The vase life of flower is the number of days that the flower remains in an acceptable state until the end of consumer use (Maree and van WYK, 2010).

Cut flowers deteriorate rapidly and can become unacceptable because of symptoms such as sepal yellowing, petal blueing, petal wilting and abscission, loss of flower bud opening, and peduncle bending (bent neck). These symptoms are caused by different processes and mechanisms occurring during flower senescence. Symptoms of loss of fresh weight of flower tissue, such as drying and shrivelling, occur in the final phase of senescence. Cut flowers may also lose water during vase life, indicating a loss of membrane integrity and increased permeability and leakage. The increase in the leakage of solutes from cells is related to loss of turgidity and

visible wilting. It has been suggested that this increase in solute leakage is due to increased permeability of the membrane (Salunkhe *et al*., 1989).

Postharvest physiological processes in rose flowers occur in the leaves, stems, flower buds, and the leaflet peduncle, or scape, connecting the bud to the stem. The flower bud and peduncle are dependent on the water supply through the stem. Therefore, a major factor in senescence is the water status of the stem, which is a result of water uptake through the stem base. Loss of water uptake will lead to a decline in stem water potential. An improvement in the water balance may be achieved in some cases through an increase in water uptake and water conductivity by recutting the stem base under water, by water acidification and by degasification of water (Zieslin, 1989).

The postharvest life of roses often comes to an end due to a loss of peduncle rigidity, known as bent neck. The development of this symptom is considered to be caused by vascular occlusion, which inhibits water supply to the flowers (Van Doorn, 1997). Vascular occlusion is caused by a number of factors such as bacteria (Van Doorn *et al*., 1989), and air emboli (Van Doorn, and de Witte, 1991). Growth and rigidity of rose peduncles are also affected by gibberellin and auxins, and the anatomical development and lignification of the peduncle is correlated with the activity of phenylalanine ammonia lyase in the peduncle tissue (Zieslin, 1989).

1.7 Factors affecting vase life

Consumers usually select flowers for their appearance, quality and vase life. As discussed above, these qualities are influenced by pre-harvest and postharvest conditions.

1.7.1 Respiration and substrate supply

Because of a general limitation in sources of energy, the longevity of cut flowers is shorter than that attached to the parent plant. Respiration is the process of living cells which generates energy for overall metabolism. The rate of respiration in many cut flowers is high during opening, and then declines during senescence. However, there is a second increase in respiration for a short period, followed by a

final decline. The second peak in the respiration indicates the last phase of senescence. It is similar to the climacteric rise seen in the respiration of many fruits (Wills *et al*., 1998).

During flower maturation, the respiration gradually declines, which may be caused by an insufficient supply of sugars for respiration (Goszczynska *et al*., 1990). Carbohydrate is used as a respiratory substance and maintains a potential osmotic pool in petals. Carbohydrates are important in many growth processes in plants; they are important for turgor maintenance, essential to plant flowering (Bernier *et al*., 1993) and an important energy source for flower opening (Marissen and LaBrijn, 1995).

Flowers usually have high rates of respiration through glycolysis and the tricarboxylic acid (TCA) cycle based on sugar translocation from the leaves. For cut flowers, the preservative solution, which is often supplied in sachets with bought flowers and can be used in the vase water, contains sucrose or a carbohydrate source to help maintain the respiration rate and to extend longevity (Wills *et al*., 1998). The application of exogenous sugar has been recommended for delaying the onset of senescence. The principal effect of applied sugars is to maintain the structure and function of the mitochondria (Lineberger and Steponkus, 1976). Sucrose added to the vase solution improves the development of flower colour and size, prevents senescence, and decreases the osmotic potential of cell and membrane degradation (Kuiper *et al*., 1996).

1.7.2 Effect of pre-harvest condition on vase life

Good quality and the long life of cut flowers depend, to some extent, on conditions during cultivation. Light intensity and temperature during growth have an effect on the carbohydrate levels of many cut flowers.

Light intensity influences the efficiency of photosynthesis due to its effect on the leaf photosynthetic rate (Nowak and Rudnicki, 1990). Fjeld *et al*. (1994) report that the vase life of different cultivars of cut roses varies due to differences in response to supplemental lighting. In their study, total light energy during the growth period affected the vase life of flowers. In general, the cultivation of roses under low light conditions often reduces stem quality and increases the flower abortion rate. For

a given light condition, day and night temperatures can also influence flowers' stem quality (Van Labeke *et al*., 2001). Also, changes in light intensity affect the leaf photosynthetic rate (Sevelius *et al*., 2001). The pigmentation and colour of rose flowers are strongly influenced by environmental conditions during cultivation. Blueing of the red and pink cultivars is largely attributable to a decrease in the anthocyanin content of the petals during periods of low light intensity and high temperature under cultivation. Low night and day temperatures are correlated with the accumulation of pigments and are often accompanied by blackening of petals. Moreover, low temperatures generally affect the growth from seedling to reproductive stage in most plant species (Pandya and Saxena, 2003).

High temperatures reduce carbohydrate levels and reduce the vase life of freesias, tulips, irises and carnations (Torre *et al*., 2001). High relative humidity during growth can cause a high incidence of bent neck and leaf drying, decreasing the vase life of cut flowers. High relative humidity (91%) reduced the vase life of both 'First Red' and 'Golden Gate' roses (Slootweg *et al*., 2001). Moreover, high air humidity slightly affected the vase life of 'Frisco', 'Golden Gate', 'Dream' and 'Kardinal' roses, while it reduced the vase life of 'Orange Unique', 'Miracle', 'Prophyta' and 'Amadeus' (Mortensen, 2001).

In general, the efficiency of photosynthesis and production rates are increased with increased CO_2, particularly in low light intensity environments. Increasing CO_2 concentration in greenhouses can increase plant tolerance to water deficit stress. The yield and quality of rose flowers grown in greenhouses have been increased by maintaining the CO_2 concentration (Mortensen, 2001). However, high CO_2 can cause leaf wilting by an accumulation of starch in leaves and the deformation of chloroplasts (Kramer, 1981).

1.7.3 Carbohydrate and flower quality

During petal growth, starch is hydrolysed and also used for cell maintenance. During petal cell expansion, starch is decreased and soluble sugar is increased in the petals. Carbohydrates are substrates for cell wall components synthesis (Mayak *et al*., 2001). Reducing sugars, such as glucose and fructose, are the main sugars in petals. In roses, fructose has been reported as the predominant carbohydrate during the opening

of flowers (Ichimura *et al*., 1999). The level of fructose and glucose is reported to increase rapidly in petals at the time of flower opening and continues to increase until the petals are about to drop (Van Doorn, 2001). Sucrose transferred from other organs to petals, is metabolized into glucose and fructose and accumulates in petals (Yamada *et al*., 2007). Myo-inositol is a precursor of structural carbohydrates, a composition of membrane, and a reserve substance; it can be found in all parts of roses. The role of myo-inositol in the petal growth of roses is unclear. However, exogenous myo-inositol at a high concentration inhibits water uptake and flower opening (Ichimura *et al*., 1999).

In roses, the leaves can act as a storage pool for carbohydrates, which are transported to the flower bud during vase life (Marissen, 2001). Sugars are transported from leaves to flower bud via the phloem. Carbohydrate transport appears to be extremely important – a good flower opening cultivar was able to transport more carbohydrates from the leaves and stem than a poor flower opening cultivar, as reported by Marissen and La Brijn, (1995). However, accumulation and transportation of sugars may relate to other factors. Carbohydrate concentrations and fluxes in the leaves and petals of roses may not be indicative of the vase life characteristics alone (Marissen, 2001).

Van Doorn (2001) reports that soluble sugars in the petals are still high at the time of senescence. It is possible that sugar levels in the cytosol become limiting for vase life, while still having high sugar concentrations in the vacuole. Moreover, Kumar *et al*. (2008) suggest that sugar concentrations are still high when petals reach the early stages of senescence. There are many reasons for this; it may in fact be that the measurement method is not suitable because various tissues in a petal are at different stages of senescence; sugar concentrations found in the cytoplasm and the vacuole are different; sugars are still formed or moved to petals.

The vase life of lily flowers depends on the number of competitive sinks within the inflorescence after harvest. The role of carbohydrates in the postharvest life of the lily is important (van der Meulen-Muisers *et al*., 2001). Arrom and Munne-Bosch (2012) report that endogenous glucose contents increased during flower opening and decreased during senescence in all floral organs, while sucrose contents increased in the outer and inner sepals. The sucrose content in the sepals gradually

increased with bud development, but remained at relatively low levels for all stages (van der Meulen-Muisers *et al*., 2001). The carbohydrates available for an individual floret are used for maintenance respiration, an increase in structural dry weight and to maintain an osmotic pool in the petal cells of the floret. There was no difference in final dry weight between buds at different positions when the amount of available carbohydrates was varied during the growth of lily flower buds (van Meeteren *et al*., 2001).

1.7.4 Xylem Occlusion

Water stress during vase life is one of the possible causes of shortened vase life. The blockage of water flow in a xylem may be a cause of water deficit (Nijsse and van Meeteren, 2000). Maintaining optimum balance between water uptake and transpiration is an important part of maintaining vase life. Changes in water relations generally have an effect on vase life. Factors affecting xylem occlusion can be divided into physiological stem plugging and microbial-induced stem plugging.

Physiological stem-plugging relates to enzymatically mediated plugging resulting from injured cells at the cut ends of stems. Wounding may induce physiological processes involving catechol oxidase and peroxidase (van Doorn and Cruz, 2000; Vaslier and van Doorn, 2003; Loubaud and van Doorn, 2004, He *et al*., 2006). Van Doorn (1995) reports that physiological blockages can be divided into three groups: (1) exudation of substance at the cut surface (latex or gums); (2) deposition of lignin and tannin, and deposition of gum; and, (3) formation of a balloon-like outgrowth of cells around a xylem conduit called tyloses.

Water uptake may also be limited by high concentrations of bacteria in the vase water. Vascular blockage in cut flowers has been studied during investigations into the role of microorganisms on vase life. The role of microorganisms in the vase life of cut rose flowers has also been studied by adding antimicrobial compounds or bacterial cultures into the vase water (van Doorn *et al*., 1989; Zagory and Reid, 1986). In general terms, the onset of water stress symptoms, such as leaf wilting and bending of the stem was delayed by adding antimicrobial compounds but could be made more severe by including bacteria in the vase water. Antimicrobial compounds are sometimes included in ‘flower foods’ and are generally able to reduce the number of

bacteria both in the solution and in the stems of the rose flowers (van Doorn *et al*., 1990). Thus, vascular occlusion due to bacteria can be reduced in some instances by applying germicides such as silver nitrate, aluminium sulphate and 8-hydroxyquinoline sulphate, all of which have been used in commercial preservatives (Ueyama and Ichimura, 1998).

However, there is no research at present into factors that may influence the xylem occlusion of flowers in mixed flower bouquets. Previous research has studied xylem occlusion in single varieties of flowers, and the dynamics of mixed flowers may result in unique conditions that influence this problem.

1.8 The role of microorganisms

In 1989, van Doorn *et al*. reported that there were five *Pseudomonas spp*. and one *Alcaligenes spp*. in the vase water of 'Sonia' roses and they also found these bacteria in the xylem vessels in stems. Moreover, *Pseudomonas* and *Enterobacter spp*. were always found at the cut surface and in the xylem of the rose (de Witte and van Doorn, 1988). The *Pseudomonas* species were found in more than 70% of the total bacteria population on the cut surface and xylem, and *Enterobacter* species (mainly *Ent. agglomerans*) for less than 10%. Moreover, *Acinetobacter*, *Aeromonas*, *Bacillus*, *Citrobacter* and *Flavobacterium* were also sometimes present (van Doorn *et al*., 1991a). Identification of bacteria were made on samples from cut flowers obtained from different environments, and 41 different bacteria species were found, 12 species of which were *Pseudomonas* (Kates *et al*., 1991). The presence of particular bacteria in vase water (such as *Bacillus*, *Enterobacter*, *Pseudomonas*) and fungi during vase life, may be correlated with the specific host plant. However, the increase in microorganism populations in vase water may relate more to the ecological conditions in the vase rather than the microorganisms initially present on the stems (Put, 1990). Microorganisms found in or on rose stems, are shown in Table 1.2. However, there was a lack of information on bacteria found in the vase water of cut lilies.

Bacteria in the xylem were found to be higher in number close to the cut end (Van Doorn *et al*., 1991). Bacteria located in the basal 5 cm of the stem may limit water uptake in cut rose stems due to a correlation between a decrease in hydraulic

conductance and the high number of bacteria (Van Doorn and de Witte 1991.) Bleeksma and van Doorn (2003) report that bacteria in vase water increases over time and accumulates at the cut surface and inside the xylem, where they block water uptake. Once their numbers exceed a particular threshold, the rate of water uptake becomes lower than the rate of transpiration, which results in a low water potential. In carnation stems, which were held in water for seven days at 20^{o}C, there occurred a low hydraulic conductance and a high number of bacteria were found in the basal 5 cm stem segment. Bacterial populations developing in the stems of cut carnation flowers during vase life were shown to lead to vascular occlusion but this apparently had little effect on flower longevity (Van Doorn *et al*., 1991).

Table 1.2: Microorganisms found in or on rose stems (Modified from Kates *et al*., (1991) and Laird, (2005)).

Microorganisms	Gram	Kim *et al*., 1997	Van Doorn and de Witte, 1997	Van Doorn and de Witte, 1991	Put, 1990	Van Doorn *et al*., 1990	Put and Jansen, 1989	De Witte and van Doorn, 1988	Ford, Clark and Stinson, 1961	Kates *et al*., 1991
Achromobacter butyri	Negative								√	
Achromobacter delicatum	Negative								√	
Achromobacter formosum	Negative								√	
Achromobacter liquefaciens	Negative								√	
Achromobacter nitroficans	Negative								√	
Achromobacter pestifer	Negative								√	
Acinetobacter calcoaceticus	Negative									√
Aeromonas hydrophila	Negative	√								√
Aeromonas sobria	Negative									√
Alcaligenes spp.	Negative		√		√				√	
Alcaligenes denitrificans	Negative									√
Alcaligenes faecalis	Negative								√	
Alcaligenes marshall	Negative								√	
Arthrobacter spp.	Positive									√
Bacillus spp.	Positive									√
Bacillus adhaerens	Positive								√	
Bacillus aurantius	Positive								√	
Bacillus cereus	Positive				√					
Bacillus subtilis	Positive				√		√			
Bordetella spp.	Negative									√

Table 1.2 (Conc.): Microorganisms found in or on rose stems (Modified from Kates *et al*., (1991) and Laird, (2005)).

Microorganisms	Gram	Kim *et al*., 1997	Van Doorn and de Witte, 1997	Van Doorn and de Witte, 1991	Put, 1990	Van Doorn *et al*., 1990	Put and Jansen, 1989	De Witte and van Doorn, 1988	Ford, Clark and Stinson, 1961	Kates *et al*., 1991
Citrobacter spp.	Negative		√							
Citrobacter freundii	Negative				√					√
Enterobacter spp.	Negative		√				√			
Enterobacter aerogenes	Negative									√
Enterobacter agglomerans	Negative				√		√			
Enterobacter agglomerulans	Negative									√
Enterobacter amnigenus	Negative									√
Enterobacter cloacae	Negative				√					√
Escherichia coli	Negative								√	√
Flavobacterium spp.	Negative									√
Flavobacterium aurantiecum	Negative								√	
Klebsiella oxytoca	Negative	√								√
Klebsiella ozaena	Negative									√
Klebsiella pneumoniae	Negative									√
Klebsiella rhinoscleromatis	Negative									√
Micrococcus auranticus	Positive								√	
Micrococcus candidus	Positive								√	
Micrococcus ochraceus	Positive								√	
Micrococcus sphaerroides	Positive								√	
Pseudomonas spp.	Negative	√	√	√	√	√	√	√	√	

Table 1.2 (Conc.): Microorganisms found in or on rose stems (Modified from Kates *et al*., (1991) and Laird, (2005)).

Microorganisms	Gram	Kim *et al*., 1997	Van Doorn and de Witte, 1997	Van Doorn and de Witte, 1991	Put, 1990	Van Doorn *et al*., 1990	Put and Jansen, 1989	De Witte and van Doorn, 1988	Ford, Clark and Stinson, 1961	Kates *et al.*, 1991
Pseudomonas acidovorans	Negative									√
Pseudomonas aeruginosa	Negative			√				√		√
Pseudomonas arvilla	Negative								√	
Pseudomonas cepacia	Negative							√		√
Pseudomonas acidovorans	Negative									
Pseudomonas desmolyticum	Negative								√	
Pseudomonas fluorescens	Negative	√			√	√	√			√
Pseudomonas luteola	Negative									√
Pseudomonas maltophilia	Negative							√		√
Pseudomonas nonliquefaciens	Negative								√	
Pseudomonas paucimobilis	Negative									√
Pseudomonas pickettii	Negative									√
Pseudomonas putida	Negative						√	√		√
Pseudomonas stutzeri	Negative							√		√
Pseudomonas testosteroni	Negative									√
Pseudomonas vesicularis	Negative							√		√
Serratia liquefaciens	Negative									√
Staphylococcus spp.	Positive									√
Vibrio alginolyticus	Negative									√
Vibrio fluvialis	Negative									√

Table 1.2 (Conc.): Microorganisms found in or on rose stems (Modified from Kates *et al*., (1991) and Laird, (2005)).

Microorganisms	Gram	Kim *et al*., 1997	Van Doorn and de Witte, 1997	Van Doorn and de Witte, 1991	Put, 1990	Van Doorn *et al*., 1990	Put and Jansen, 1989	De Witte and van Doorn, 1988	Ford, Clark and Stinson, 1961	Kates *et al*., 1991
Vibrio parahaemolyticu	Negative									√
Candida albicans										√
Candida tropicalis										√
Trichosporon beigelii										√
Torulopsis candida										√
Rhodotorula glutinis										√
Fungi										√

1.9 Preservative solutions

Floral preservatives (vase/holding solution) are typically used by consumers to extend cut flower vase life. The use of preservatives considerably increases the vase life of flowers (Celikel and Karacaly, 1995). Preservative solutions for cut flowers usually consist of germicides or bactericides, a carbohydrate, surfactants, acidifiers (such as citric, benzoic, or ascorbic acid) (Teixeira da Silva, 2003).

The role of exogenous sugars for extending the vase life is well known. Sugar from vase solutions accumulates in petal tissues, improving their osmotic potential and enhancing the carbohydrate pool for growth and respiration (Van Doorn *et al*., 1991b; Kuiper *et al*., 1995). Flowers supplied with a glucose or sucrose solution have a longer vase life, associated with a longer blooming time. Flowers supplied with a sugar solution develop complete blooming, which is not always the case when supplied with water alone. This prolonged vase life seems to be associated with a constant fresh weight and regular increase in dry matter (Paulin, 1986). In addition, exogenous sucrose, which enters into the composition of all keeping-solutions, plays an important role by providing a readily available substrate for respiration, by slowing the degradation of proteins (Mayak *et al*., 1973).

However, sugars in the vase solution increase bacterial growth, which may result in xylem blockage and possibly other detrimental effects. Therefore, an antimicrobial compound is usually added to sugar solutions. Antimicrobial compounds could control bacterial growth, retain water uptake and delay senescence; these compounds are: metal salts, quinoline compounds, ammonium compounds and compounds including chlorine (Van Doorn, 1990).

Generally flower food always consists of antimicrobial compounds for the prevention of bacterial growth in vase water. Antimicrobial compounds in the vase solution were found to reduce the number of bacteria both in the solution and in the stems of rose flowers (Van Doorn and de Witte, 1991.)

The principal chemical preservative ingredients in commercial floral preparations are quinoline salts. The quinoline compounds have been used in medicine and industry for many years, principally as fungicides (Marousky 1980).

More recently, Hydroxyquinoline sulfate (HQS) and Hydroxyquinoline citrate (HQC) have been widely used as constituents of flower preservatives (Kofranek and Halevy, 1972).

Chlorine compounds, including sodium hypochlorite, calcium hypochlorite and dichloroisocyanuric acid (DICA), are commonly used in vase solutions (Knee, 2000). The action of chlorine involves oxidation of proteins in cell membranes and the protoplasm (Xie *et al*., 2008).

Citric acid acts as a pH regulator and is used in many vase solution formulations to generally increase water conductance in the xylem of cut flowers and reduce bacteria populations in the vase water (Darandeh and Hadavi, 2012). A citrate-phosphate buffer at pH 3.0 has also been used for roses, and prevented bacterial growth and vascular blocking (van Doorn *et al*., 1990). Malic acid is another acid which can be used and has also been shown to prevent vascular blockage by reducing bacterial populations in vase solutions (Kazemi *et al*., 2010).

1.10 Essential oils

Essential oils are organic natural compounds that have strong antimicrobial properties against some pathogens because of high levels of phenolic compounds such as carvarol, thymol and eugenol. Essential oils are safe and environmentally friendly (Solgi *et al*., 2009). They are good alternatives to the chemicals currently used as antimicrobials.

Many plant species themselves produce essential oils that contain complex mixtures of secondary metabolites that play a role in chemical defence (Teixeira da Silva, 2003). Commercial essential oils from plants are often mixtures of several components; some of those components are oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage and vanillin, all of which have been shown to express antimicrobial effects (Tajkarimi *et al*., 2010).

Essential oils and other plant extracts are normally the components that are responsible for antimicrobial activities in herbs and spices. These plant compounds, including glucosides, saponins, alkaloids, tannins, organic acids, essential oils etc., are

vital parts of the plant defence system against microbial infection (Bajpai *et al*., 2008; Tajkarimi *et al*., 2010). An important general antimicrobial property of essential oils and their components is their hydrophobicity, which enables them to partition with the lipids of bacterial cell wall, cell membrane and mitochondria, causing increased permeability of these membranes. Leakage of ions from these membranes can lead to bacterial cell death (Solorzano-Santos and Miranda-Novales, 2011).

Some of the antimicrobial activities of essential oils (components of spices and herbs) are shown in Table 1.3.

Table 1.3: Major plant species with antimicrobial activity and their active components (Source: Tajkarimi *et al*., 2010).

Category	*Species*	*Plant part*	*Major active component*	*Bacterial inhibition (%)*
Herbs	Basils, sweet (*Ocimum basilicum*)	Leaves	Linalool/methyl chavicol	< 50
	Oregano (*Origanum vulgare*)	Leaves/ flowers	Cavacrol/thymol	75-100
	Rosemary (*Rosmarinus offinicalis*)	Leaves	Camphor/1,8-cineole/borneol/camphor	75-100
	Sage (*Salvia officinalis*)	Leaves	Thujone, 1,8-cinole/borneol/camphor	50-75
	Thyme (*Thymus vulgares*)	Leaves	Thymol/carvacol	75-100
Spices	Allspice, pimento (*Pimenta diocia*)	Berry/leaves	Eugenol/β-caryophyllene	75-100
	Cinnamon (*Cinnamonum zeylanicum*)	Bark	Cinnamic aldehyde/eugenol	75-100
	Clove (*Syzgium aromaticum*)	Flower bud	Eugenol	75-100

* Bacterial inhibition (%)-The process of prohibiting, restraining, or hindering the growth of bacteria, including the inhibition of enzyme activity within the bacteria.

There is a large amount of literature about the investigation of the use of essential oils as alternative antimicrobials. For example, using essential oils such as garlic oil in fruit and vegetable washing solutions reduced the number of bacteria which decreased the risk of pathogen contamination in fruit and vegetables and the washing solutions (Solorzano-Santos and Miranda-Novales, 2011). In addition, applying 500 mg/L thyme oil into nutrient agar showed 100% control of fungal species such as *Botrytis cinerea*, *Rhizopus stolonifer* and *Alternaria alternate* (Plotto *et al*., 2003).

1.11 Weak organic acids

Generally, organic acids appear in vegetable and animal substrates. An organic acid is an organic compound with acidic properties and containing carbon, the same as organic compounds. Organic acids found in foodstuffs are shown in table 1.4. Many researchers have reported that organic acids have the efficacy of antimicrobials and are used in food industry as food preservatives (e.g. Theron and Rykers Lues, 2011).

The cell membrane of bacteria becomes damaged when the cell is exposed to severe pHs. The leakage of H^+ and OH^- ions into the cell where enzyme and nucleic acid are denatured, leads to the cell's death. The degree of dissociation of weak organic acids depends on the pH of the environment. If there is an excess of H+ ions in acid solutions, the equilibrium moves towards the undissociated form. The undissociated acid is lipid soluble and can pass through the cell membrane where the dissociated ions cannot. Once undissociated acids enter the cell, the undissociated molecule can dissociate to slightly acid conditions. Next, the cell pumps out excess H^+ ions, then the internal cell pH is decreased and has an effect on enzyme activity and nucleic acids. Then the cells become dead (Garbutt, 1997). Entry of weak acids in cells relies on them being initially in the undissociated form, therefore, weak organic acids require a buffer system for control the constant pH to be effective.

The food industry widely uses organic acids as a type of food preservative in food and beverages products. The common weak organic acid preservative consists of benzoic, acetic, sorbic and propionic acids, together with sulfide (Hazan *et al*., 2004). Due to organic acids being natural products, they are safe and environmentally friendly.

Table 1.4: Organic acids found in foodstuffs (Source: Theron and Rykers Lues, 2011).

Organic acids	***Foodstuff***
Acetic acid	Vinegar
Benzoic acid	Cranberries, prunes, cinnamon, ripe cloves, apples
Butyric acid	Butter
Citric acid	Citrus fruit, blackcurrants, strawberries
Formic acid	Citrus essential oil
Lactic acid	Sour mild products
Malic acid	Apples, cherries, plums
Oxalic acid	Tomato
Tartaric acid	Grape juice

1.12 Aim and objectives

1.12.1 Aim

The aim of this research is to understand some of the factors affecting the vase life of cut flowers in mixed flower bouquets.

1.12.2 Objectives

- To develop an index for the assessment of vase life: degree of bud opening, degree of flower quality, degree of leaf quality

- To study the relationship between, and effect of, sugar content in petals or sepals, on the vase life of different varieties of roses and lilies

- To identify, quantify and profile microbial populations in both vase life water and plant material in single variety bouquets and mixed bouquets
- To investigate chemical exudates from plant stems that may influence vase life
- To consider new methods to control microbial growth in vase water.

1.13 Thesis structure

This thesis consists of seven chapters. Chapter one reviews the literature of cut flowers. First, the cut flower market in the UK and trend of mixed flower bouquets are described, followed by a brief history of roses and lilies. Then, factors affecting the postharvest life of cut flowers are described followed by listing postharvest treatments which influence the vase life of cut flowers.

Even though relations between carbohydrates and vase life have been studied in many flowers, the role of sugars on the longevity of cut flowers is not clear. Chapter two creates a quality standard of nine varieties of cut rose and two varieties of lily. Changes of sugars during seven days of vase life are described, in relation to roses and lilies, and mixtures of these flowers.

In the past, bacteria in vase water have been studied for their effect on the vase life of many flowers, but this has not included an investigation into the effects of mixtures of flower types. Chapter three describes bacteria in the vase water of the 'Akito' rose and 'Tiber' lily, and mixtures of these. Differences in the number of stems were studied for their effect on increases in the number of bacteria. Moreover, the number of bacteria on the cut surface, outer stem and in the xylem were investigated in the 'Akito' rose and 'Tiber' lily.

There is little research that has studied the effect of chemical exudates from stems in vase water on the longevity of flowers of other species, with particular reference to mixtures. Chapter four describes the effect of chemicals that were exudates from the stems of the 'Akito' rose and 'Tiber' lily on the vase life of each flower.

Although antimicrobial agents have been studied for many years for their application to cut flowers, this project intends to look for new antimicrobial agents that are safe for humans and create no risk to the environment. Chapter five describes some early investigations into the possibility of using essential oils and weak organic acids as antibacterial agents for the control of bacterial growth in vase water.

Chapter six is a discussion and gives conclusions. This chapter is an integration of previous results and offers recommendations for future research.

CHAPTER TWO

Variation in sugar content and vase life of cut roses and lilies

2.1 Introduction

Consumers themselves use freshness and senescence symptoms as criteria of a flower's quality. In the cut flowers industry, vase life is stated as a guarantee for consumers. Much previous research has involved the study of how to extend postharvest life and investigated factors affecting the vase life of each flower. However, there is less research that has studied how to extend the vase life of mixed flowers bouquets. Flowers arranged in bouquets often consist of various characteristics such as a single-type flower (e.g. rose, gerbera, sunflower) and inflorescence-type flower (e.g. lily, gladiolus, carnation). Further, consumers sometimes buy flowers or bouquets on impulse. Thus the immediate appearance (i.e. the length of stems, colour, shape of flowers and leaves, packaging and flower's presentation) is of importance (Maree and van WYK, 2010).

Sugars are not only an important energy source and structural component, but they are also central regulatory molecules controlling metabolism, cell cycle, development, and gene expression in prokaryotes and eukaryotes (Kumar *et al*., 2008).

The role of soluble carbohydrates such as fructose, glucose, sucrose and myo-inositol, has been studied for their effect on the quality and vase life of many flowers. Ichimura *et al*., (2002) found the content of soluble carbohydrates in cut flowers is related to the length of vase life. The relation between vase life and carbohydrate pool in leaves and flower bud may in part be related to the levels of supplementary lighting and growth temperature (Marissen, 2001). A sugar supply in the vase solution increases vase life, due to the sugars being used as a supplementary substrate for respiration, maintenance, synthesis and osmoregulation, therefore senescence is delayed. For flowers opening, soluble carbohydrates are required as a substrate for respiration and synthetic materials (Kaltaler and Steponkus, 1976). In roses, the leaves

can act as a storage pool for carbohydrates, which are transported to the flower bud during vase life (Marissen, 2001).

Not only are single flower bouquets popular but also mixed flowers bouquets are becoming increasingly more popular. Due to the aesthetics of having various different cut flowers in the same bunch, these attract consumers. However, optimum harvest stage of each flower is difference and these influence the vase life of each flower. Therefore, studies described here on the differences in vase life among cultivars and related to sugar changes may give a basic knowledge on how to extend the vase life of mixed flowers bouquets.

The objective of this study is to investigate longevity and changes of sugars during the vase life of nine varieties of cut roses and two varieties of cut lilies. Investigations into differences in sugar content were made among various cultivars of roses and different positions in the same inflorescence of lilies. This study also introduces a flower quality standard index for the assessment of vase life.

2.2 Material and methods

2.2.1 Plant material

Cut flowers studied in this research are some of the most popular of the commercial cultivars. Nine cultivars of cut rose (‘Akito’, ‘Blushing Akito’, ‘Sweet Akito’, ‘Glossy’, ‘Inka’, ‘Red Calypso’, ‘Tropical Amazon’, ‘Valentino’, ‘Viva’) and oriental lily (‘Mother’s Choice’ and ‘Tiber’) at commercial stage were obtained from Flamingo Holdings Company Ltd., (Gt North Road, Sandy, Bedfordshire SG19 2AJ). All the flowers were then transported to the Microbiology Laboratory at Cranfield University, UK. After that, they were selected for the same uniformity and without defects. Stem lengths of all samples were re-cut to 50 cm lengths under water using a sterile razor blade (van Doorn, 1997). Leaves on the lower one-third of the stems were stripped. Within each stem of the lily, the number of buds per stem was kept as constant as possible for determining the stem vase life.

To eliminate the effect of exogenous sugar on the investigated relations between sugar contents and vase life, two stems of cut roses or lilies were stood in

individual 2 litre vases containing 500 ml distilled water instead of using flower food. A plastic film was used to cover the top of the vases to avoid water loss (Mayark *et al*., 1973). Vases of flowers were placed in a temperature controlled vase life room at 20°C with a 12h light/dark cycle for the duration of the experiment.

2.2.2 Experiment designs

This experiment studied differences in the overall appearance of single-type flower and inflorescence-type flower.

2.2.2.1 Experiment I: Temporal changes in sugar content and overall appearance during vase life of nine cultivars of cut rose.

The experiment was a completely randomized design (CRD) with nine different cultivars. Measurements were divided into two blocks with three replications for overall appearance changes and three replications for non-structural carbohydrate content. There were two stems of cut rose per replication.

2.2.2.1 Experiment II: Overall appearance changes during vase life of two cultivars of cut lily and changes in sugar content at different positions.

The experiment was also a CRD. Measurement was divided into two groups with three replications for overall appearance changes and three replications for non-structural carbohydrate content. There were two stems of oriental lilies per replication.

2.2.3 Physiological measurements

2.2.3.1 Index development

This experiment investigated the overall appearance changes such as bud opening, leaf quality (roses) and flower quality (rose and lily) by taking photographs every day using a Nikon D3000 and then developing defined assessment criteria (indices) for each cultivar.

2.2.3.2 Bud opening standard of cut roses

The bud opening of each cultivar (‘Akito’, ‘Blushing Akito’, ‘Sweet Akito’, ‘Glossy’, ‘Inka’, ‘Red Calypso’, ‘Tropical Amazon’, ‘Valentino’ and ‘Viva’) were observed in the way they opened every day by taking photographs; then a bud opening standard was created and expressed on a scale of 1-5 as follows:

‘Akito’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Sepals start to separate from the tight bud.	Outer petals start to be released.	Half of the petals are released.	Outer petals show signs of rolling.	Flower fully open. Petals are almost rolling.

‘Blushing Akito’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Tight bud stage.	Petals start to be released.	Half of the petals are released.	Flower fully open. Half of the petals are rolling.	Almost all the petals are rolling. Anthers are just visible.

'Glossy'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Tight bud stage.	Outer petals are starting to be released.	Half petals are released.	Outer parts of petals are rolling.	Half the petals are rolling. Anthers are not visible.

'Inka'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Tight bud stage. Sepals start to be separated.	Top of outer petals started to be released.	Outer petals are 45° released.	Half of the petals are released.	Outer petals are more than 45° released.

‘Red Calypso’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Tight bud stage.	Petals start to be released.	Half of the petals are released.	Flower is fully open.	Almost all the petals are rolling.

‘Sweet Akito’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Tight bud stage with split top.	Petals started to be released.	Half of the petals are released.	Flower fully open. Outer petals are rolling.	Half the petals are rolling. Anthers are just visible.

‘Tropical Amazon’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Tight bud stage, sepals are starting to separate	Bud begins to open, some petals are released	Half of the petals are released.	Flower is fully open.	Almost all the petals are rolling. An anther is fully exposed.

‘Valentino’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Sepals released from the tight bud.	Top of outer petals start to be released.	Outer petals are released.	Half of the petals are released.	Flower fully open. Outer petals are rolling.

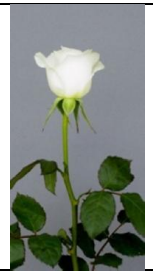
'Viva'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Tight bud stage. Sepals start to separate.	Top of outer petals are starting to be released.	Outer petals are released less than 45°.	Outer petals are more than 45° released.	Half of the petals are released.

2.2.3.3 Flower quality standard of cut roses

The flower quality of nine cultivars (‘Akito’, ‘Blushing Akito’, ‘Sweet Akito’, ‘Glossy’, ‘Inka’, ‘Red Calypso’, ‘Tropical Amazon’, ‘Valentino’ and ‘Viva’) were observed for colour changes and senescence symptoms every day by taking photographs; then a flower quality standard was created and expressed on a scale of 1-5 as follows:

'Akito'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor.	Bud starts to open but all petals and leaflets are still fresh.	Petals and foliage with the first signs of withering.	Loss of turgor in some petals and leaflets.	Pedicel with more than 20° of bent neck symptom. Petals show 10% of wilting and fading.

'Blushing Akito'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor.	Bud starts to open but all petals and leaflets are still fresh.	Loss of turgor in some petals and leaflets.	Petals with advanced signs of fading and loss of colour.	Colour is faded. All petals are rolling and 10% of them are withered.

'Glossy'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor.	Bud starts to open but all petals and leaflets are still fresh.	Outer petals with the first signs of fading.	Loss of turgor in some petals and leaflets. Petals start to pale.	Some petals getting blueing symptoms and 10% of them are withered and papery.

'Inka'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor.	Bud starts to open but all petals and leaflets are still fresh.	Petals with the first signs of withering and loss of colour.	Colour changes from red to purple. Loss of turgor in some petals and leaflets.	Outer petals show advanced signs of blueing and 10% of them are withered and papery.

‘Red Calypso’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor	Bud starts to open but all petals and leaflets are still fresh.	Petals with the first signs of wilting.	Loss of turgor in some petals and leaflets. Some petals have started to blue.	Outer petals show extreme browning and blueing and 10% of petals are withered and papery.

‘Sweet Akito’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor.	Bud starts to open but all petals and leaflets are still fresh.	Petals with the first signs of withering.	Loss of turgor in some petals and leaflets. Petals start to fade.	Colour of petals is pale and 10% of them are wilting and papery.

'Tropical Amazon'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor.	Bud starts to open but all petals and leaflets are still fresh.	Petals with the first signs of withering.	Loss of turgor in some petals and leaflets. Colour of petals is pale.	Some petals show extreme blueing and 10% of them are withered and papery.

'Valentino'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor.	Bud starts to open but all petals and leaflets are still fresh.	Loss of turgor in some petals and leaflets.	Petals with advanced signs of fading. Some petals have started to brown and blue.	The outer petals show extreme browning and blueing and 10% of them are withered and papery.

'Viva'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Petals and foliage with good turgor.	Bud starts to open but all petals and leaflets are still fresh.	Loss of turgor in some petals and leaflets.	Petals with advanced signs of fading and loss of colour.	Outer petals are pale and 10% of them are withered and papery.

2.2.3.4 Leaf quality standard of cut roses

The leaf quality of cut roses ('Akito', 'Blushing Akito', 'Sweet Akito', 'Glossy', 'Inka', 'Red Calypso', 'Tropical Amazon', 'Valentino' and 'Viva') were observed for colour changes and senescence symptoms every day by taking photographs; then a leaf quality standard was created and expressed as a scale of 1-5 as follows

'Akito'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

'Blushing Akito'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

'Glossy'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

'Inka'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

‘Red Calypso’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

‘Sweet Akito’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

'Tropical Amazon'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

'Valentino'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

'Viva'

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
Foliage with good turgor.	Foliage with the first signs of fading.	Loss of turgor in some leaflets.	Foliage with advanced signs of shrivelling and loss of colour.	Almost all leaflets are shrivelled and fading.

2.2.3.5 Flower quality standard of cut lilies

The flower quality of two lily cultivars (‘Mother’s Choice’ and ‘Tiber’) were observed for colour changes and senescence symptoms every day by taking photographs and then a flower quality standard was created and divided into 5 stages as follows:

‘Mother’s Choice’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
All buds are small and green.	First buds half open.	First and second buds are at the fully open stage.	First buds appear slightly withered.	The petals of the first buds appear papery.

‘Tiber’

Stage 1	Stage 2	Stage 3	Stage 4	Stage 5
				
All buds are small and green.	Bud half open at first bud and second bud changes to pink.	First and second buds are at the fully open stage.	First buds appear slightly withered and blueing.	The first bud appears papery. Colour of sepals turn blue and pale.

2.2.3.6 Vase life

The cut flower longevity was recorded as days of vase life from the time the flowers were placed into vases (day 0). The end of the vase life was indicated when a score of flower quality reached stage 5.

2.3.3.7 Weight of individual bud

The weights of the primary and the secondary bud of lily were measured by using balance (Model: OHAUS, TS4000D, Precision standard).

2.2.4 Biochemical assays

2.2.4.1 Sample preparation and extraction of non-structural carbohydrate

Sepals of lilies and petals and the two uppermost five-leaflet leaves of roses were snap-frozen in liquid nitrogen, and freeze-dried at -50°C for 24 hours using a freeze-drier (SCANVAC, CoolSafeTM). Samples were kept at -40°C until analyzed. Flower and leaf tissues were extracted according to Foukaraki (2008). Freeze-dried powder samples (150 mg) were each combined with 3 ml of 62.5:37.5 HPLC grade methanol: water (v/v) and mixed well. Vials were placed in a shaking water bath at 55°C for 15 min to prevent layering and then left to cool. The cooled samples were filtered through a 0.2 μm Millex-GV syringe driven filter unit (Jaytee Biosciences Ltd) and stored at -40°C until required.

2.2.4.2 Quantification of non-structural carbohydrate

Non-structural carbohydrates were analyzed according to Foukaraki (2008) using an HPLC system comprising a P580 pump and GINA 50 autosampler (Dionex, CA, USA). Petal/sepal extracts were diluted 4:10 (v/v) with HPLC grade water immediately before analysis, while leaf extracts were not diluted. The extract (20 μL) was injected into a Water Carbohydrate Analysis Column (size exclusion column of 3.9 x 300 mm, Lot No. 0065391801; Part No. WAT084038). The mobile phase was 80% (v/v) acetonitrile (filtered through a 0.4 μm filter and degassed by sparging with He for 20 min) at a flow rate of 2.0 ml min-1. Column temperature was held at 30°C using a Dionex STH column thermostat. Eluted carbohydrates were monitored by an

evaporative light scattering detector (ELSD 2420, Waters, MA, USA) connected to the Dionex system using a UCI-50 universal chromatography interface. The presence of fructose, glucose, sucrose and myo-inositol was automatically calculated against external standards using Chromeleon version 4.6 software (Dionex) (Foukaraki, 2008).

2.2.5 Statistics

Significance tests were made by analysis of variance (ANOVA) using SPSS version 16 was applied to vase life and sugar content (fructose, glucose, sucrose myo-inositol and total sugar). Mean comparisons were made using least significance difference (LSD).

2.3 Results

2.3.1 Experiment I: Temporal changes in sugar content and overall appearance during vase life of nine cultivars of cut rose

2.3.1.1 Variations in bud opening, flower quality, leaf quality and vase life of nine cultivars of cut rose.

Cut roses were studied as an example of a single flower that is used in a mixed bouquet. Changes from tight bud to the senescence stage were observed. Nine cultivars of cut roses (‘Akito’, ‘Blushing Akito’, ‘Sweet Akito’, ‘Glossy’, ‘Inka’, ‘Red Calypso’, ‘Tropical Amazon’, ‘Valentino’ and ‘Viva’) varied in the overall appearance of changes during vase life.

Bud opening was observed from tight bud stage to fully open. Characteristics of opening varied among cultivars (see bud opening standard). The scores of bud opening showed that the extent of bud opening varied between the nine cultivars. Most flowers did not completely open. ‘Red Calypso’ and ‘Tropical Amazon’ rose to reach stage 5 while other cultivars wilted without completely opening (Figure 2.1).

Changes in overall appearance were observed from the tight bud stage until end of life. When flower and/or leaf qualities of all cultivars reached stage 5, their

quality was deemed as not acceptable (i.e. end of vase life). In fact, in these experiments, the flower qualities of all cultivars reached stage 5 before the leaf qualities did (Figure 2.1). This means the flower part reaches an unacceptable stage while the leaf quality is still acceptable.

The longest vase life was 'Red Calypso' followed by 'Valentino', 'Glossy', 'Viva', 'Tropical Amazon', 'Inka', 'Sweet Akito', 'Blushing Akito' and 'Akito' (Table 2.1).

Table 2.1: Vase life of nine cultivars of cut roses.

Cultivar	Vase life (days)
Akito	4.5^{e}
Blushing Akito	6.0^{de}
Sweet Akito	6.2^{d}
Inka	6.8^{cd}
Tropical Amazon	7.0^{cd}
Viva	8.0^{cd}
Glossy	8.7^{b}
Valentino	10.5^{a}
Red Calypso	11.5^{a}

Means of the same main effect within a column followed by the same letter(s) are not significantly different at P = 0.001 probability level.

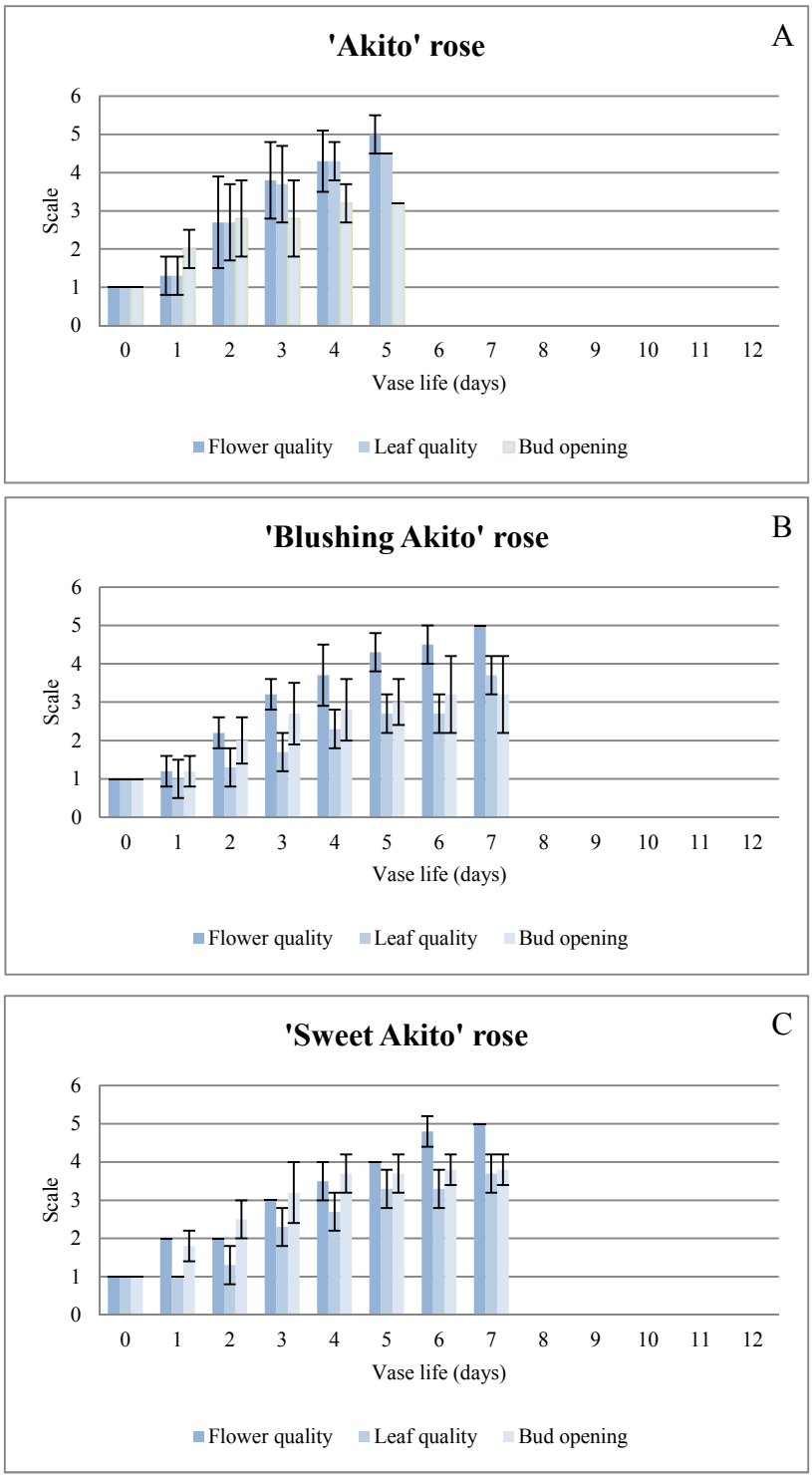


Figure 2.1: Changes in flower quality, leaf quality and bud opening of nine cultivars of cut roses during vase life, 'Akito' (A), 'Blushing Akito' (B), 'Sweet Akito' (C). Data are means of three replications, ± SD.

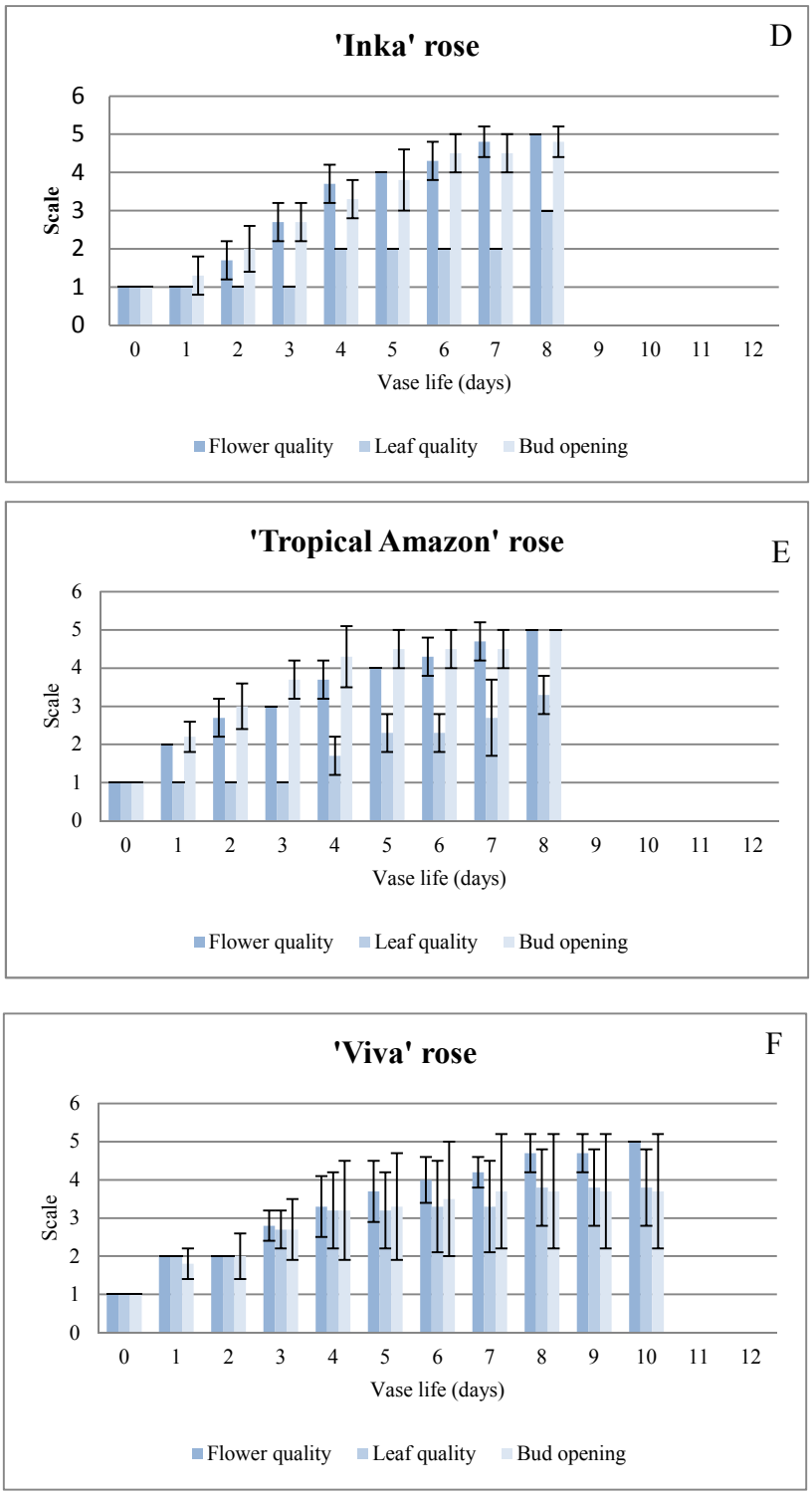


Figure 2.1 (Conc.): Changes in flower quality, leaf quality and bud opening of nine cultivars of cut roses during vase life, 'Inka' (D), 'Tropical Amazon' (E), 'Viva'(F). Data are means of three replications, ± SD.

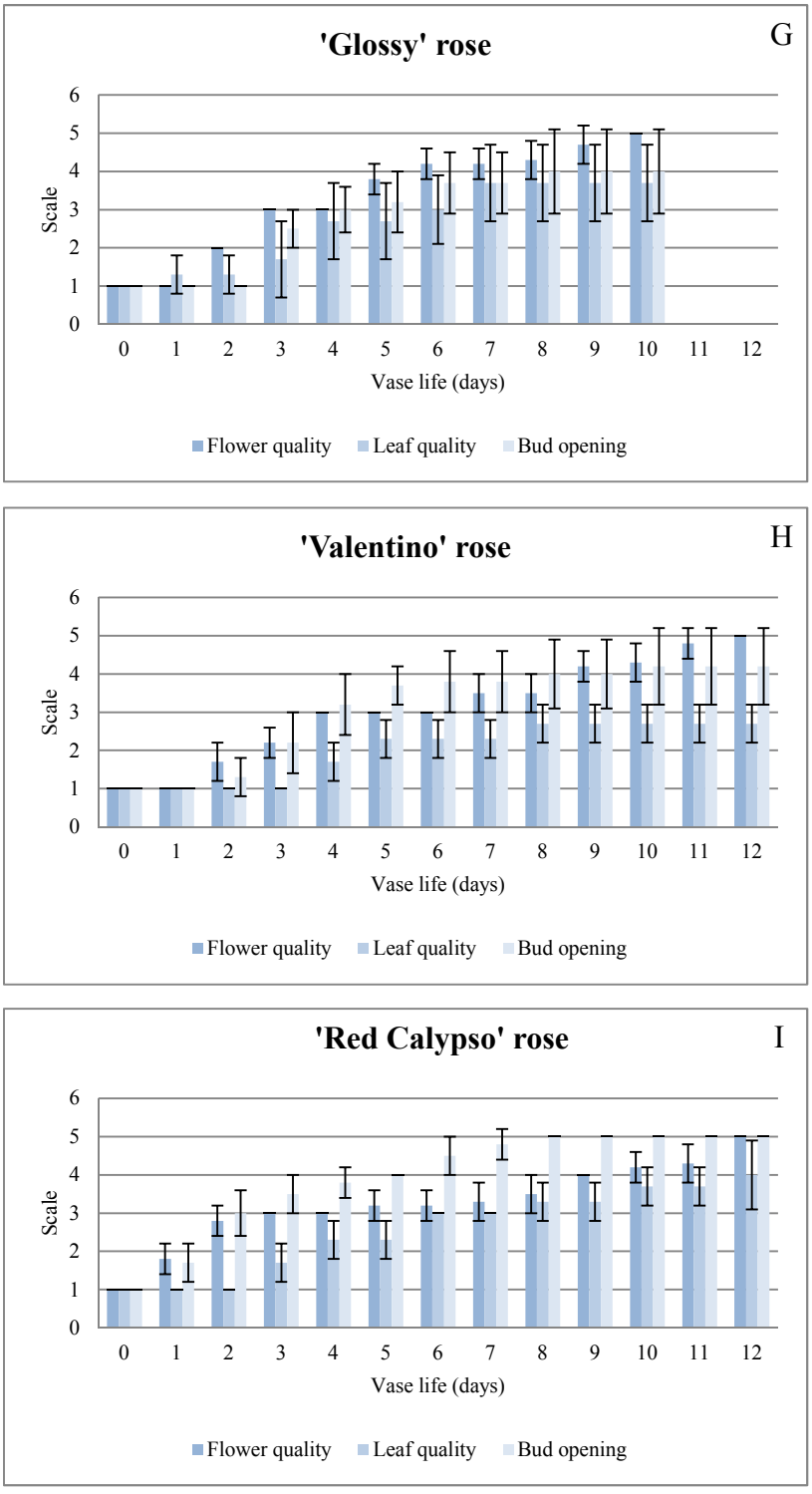


Figure 2.1 (Conc.): Changes in flower quality, leaf quality and bud opening of nine cultivars of cut roses during vase life, 'Glossy' (G), 'Valentino' (H), 'Red Calypso' (I). Data are means of three replications, ± SD.

2.3.1.2 Variation in soluble carbohydrate among nine varieties of cut roses

Due to consumer expectations that vase lives of flowers should not be less than seven days, changes in sugar content (fructose, glucose, sucrose and myo-inositol) during the first seven days of their vase life the petals and foliage were analyzed. Contents of these sugars varied among cultivars. Sugars in petals and foliage of cut roses were significantly different. In petal tissue, the concentrations of fructose, glucose, sucrose and myo-inositol in all cultivars ranged between 122.40-26.00 mg g^{-1} DW, 78.98-11.02 mg g-1 DW, 17.66-0.73 mg g^{-1} DW and 13.82-1.77 mg g^{-1} DW respectively. In the foliage, the range of concentrations was 26.14-not detected mg g^{-1} DW, 24.32-not detected mg g^{-1} DW, 77.15-5.98 mg g^{-1} DW and 26.02-9.16 mg g^{-1} DW respectively (Figure 2.2, 2.3).

Investigation into changes in sugars during the first seven days established that fructose contents in the petals decreased slightly or remained constant in some cultivars. At day 7, the fructose contents in all cultivars were not significantly different except for 'Glossy' roses when fructose contents in the leaves increased until day 4 and was not detected at day 7 (Figure 2.2 and 2.3).

For glucose, the contents of all cultivars decreased slowly across the seven days in the petals, whereas the glucose content in the leaves increased in the first stage then declined and were not detected at day 7 (Figure 2.2 and 2.3).

Sucrose contents of all cultivars declined slightly in the petals whereas they increased in the leaves except for 'Blushing Akito' which had a trend of constancy during seven days (Figure 2.2 and 2.3).

The contents of myo-inositol of all cultivars reached the highest levels at day 2 in the petals and then decreased slightly, whereas the myo-inositol contents declined throughout the seven days in the leaves. However, the myo-inositol contents in the petals of 'Glossy' and 'Valentino' roses were lower than other cultivars from day 0 to day 4 (Figure 2.2 and 2.3).

Generally, the contents of fructose and glucose were significantly higher in petal tissue compared to leaf tissue, whereas the sucrose and myo-inositol contents were significantly higher in the leaves compared to the petals.

Investigation of changes in sugars during first seven days found that total sugars of almost all cultivars slightly decreased both in petals and leaves. A comparison of total sugars in the petals and leaves among cultivars found that total sugars content of 'Glossy' was lower than in other cultivars. Total sugars content in the leaves of 'Blushing Akito' was higher than in other cultivars (Table 2.2 and 2.3).

Content of fructose, glucose, sucrose and myo-inositol in petal of all cultivar at day 7 were assessed for correlation (R^2) with vase lives of nine cultivars of cut rose. Data of correlation indicate that R^2 of all sugars were too low. Content of all sugars in petals may not relate to long-lived cultivar (Table 2.4).

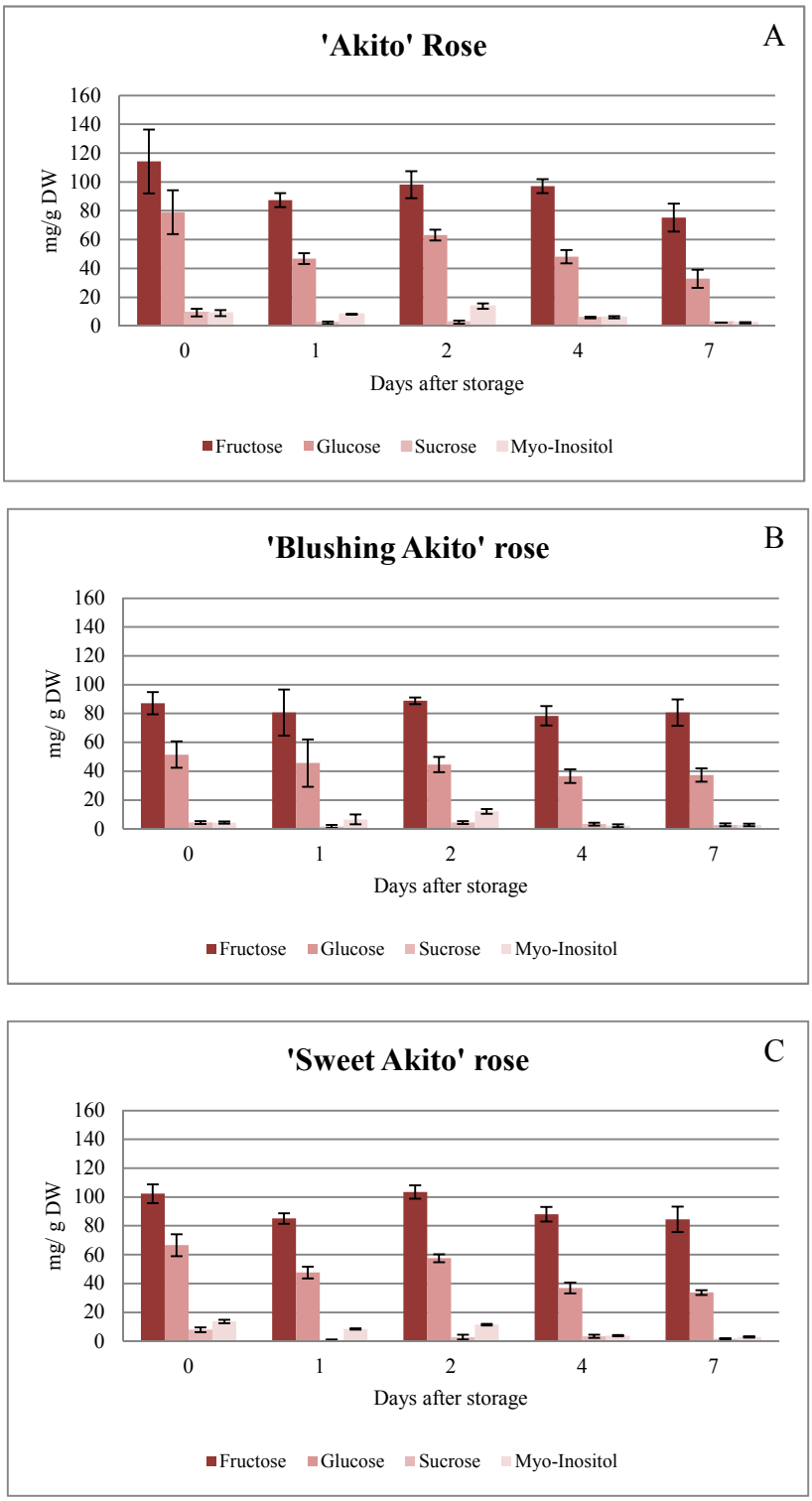


Figure 2.2: Changes in sugars in the petals of nine cultivars of cut roses during seven days of vase life, 'Akito' (A), 'Blushing Akito' (B), 'Sweet' Akito' (C). Data are means of three replications, ± SD

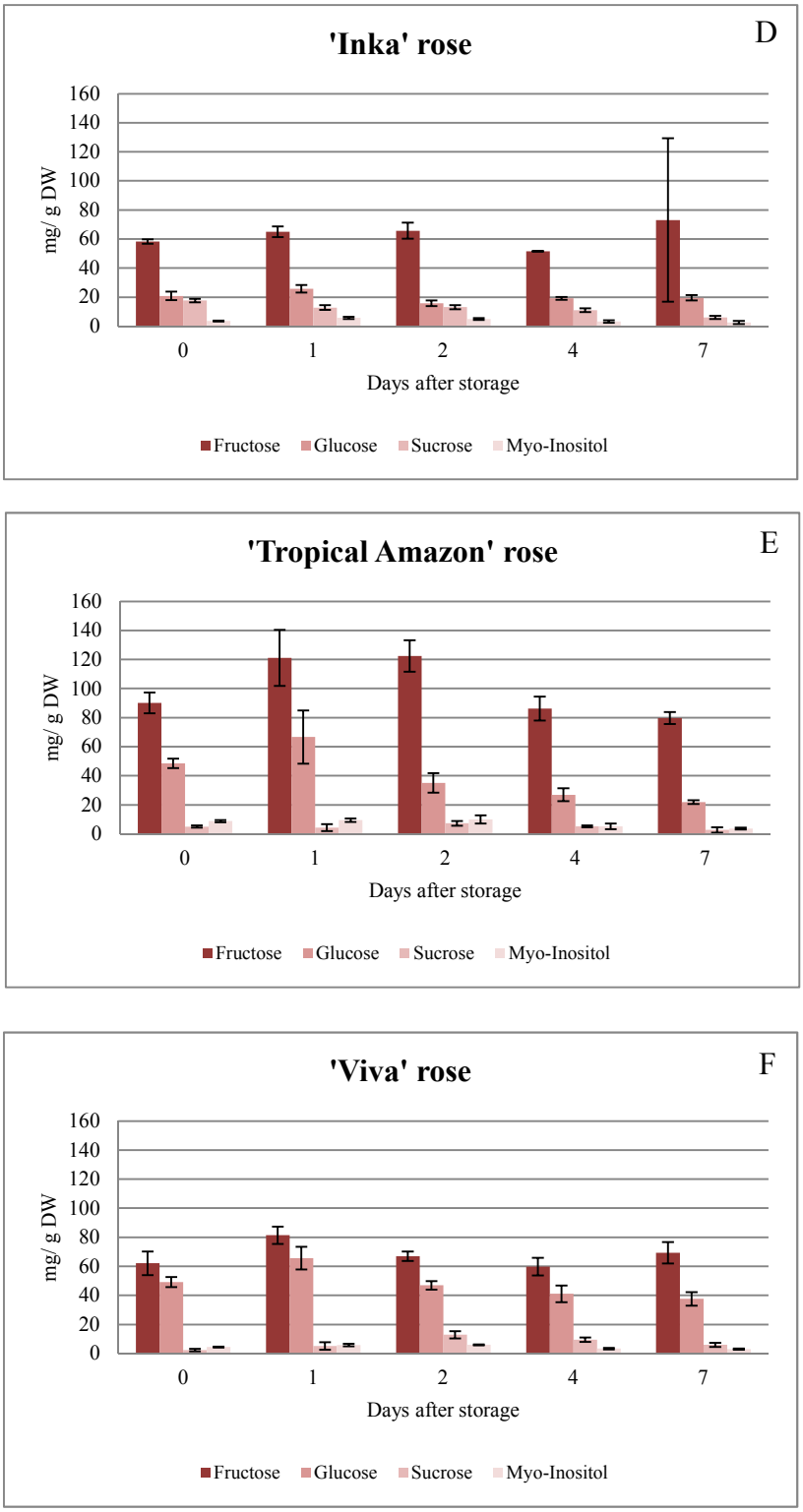


Figure 2.2 (Conc.): Changes in sugars in the petals of nine cultivars of cut roses during seven days of vase life, 'Inka' (D), 'Tropical Amazon' (E), 'Viva' (F). Data are means of three replications, ± SD.

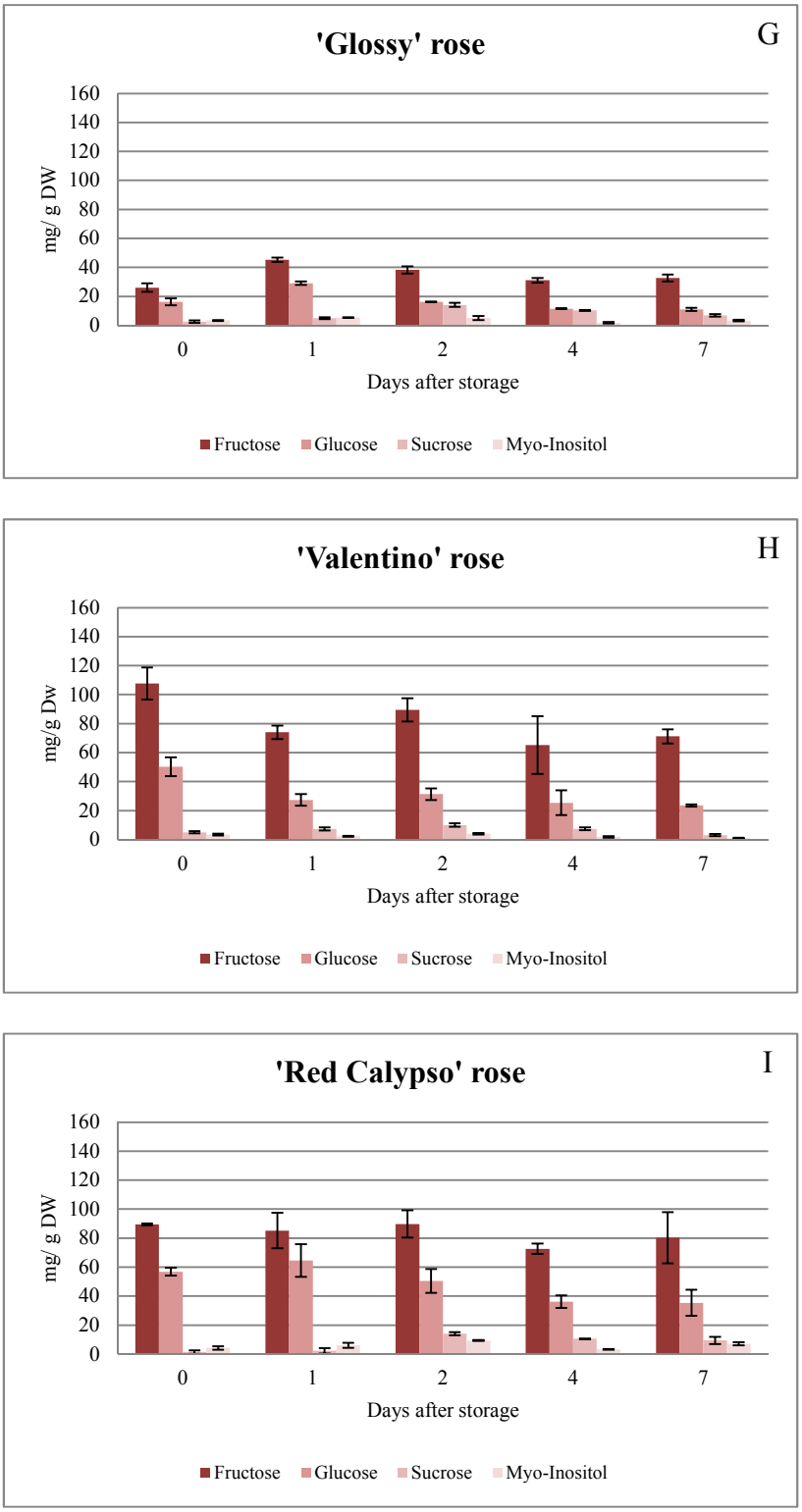


Figure 2.2 (Conc.): Changes in sugars in the petals of nine cultivars of cut roses during seven days of vase life, 'Glossy' (G), 'Valentino' (H), 'Red Calypso' (I). Data are means of three replications, ± SD.

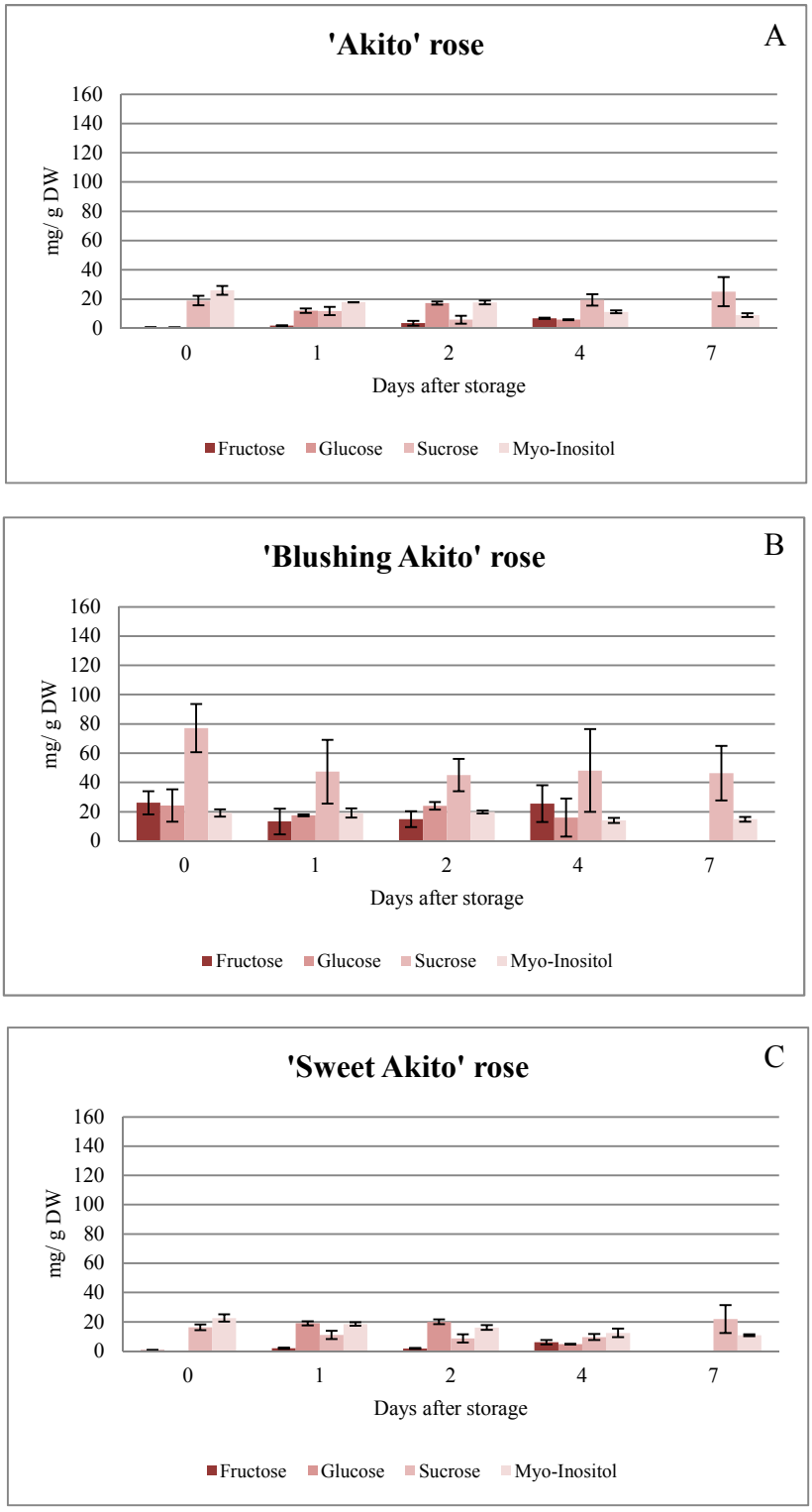


Figure 2.3: Changes in sugars in the foliage of nine cultivars of cut roses during seven days of vase life, 'Akito' (A), 'Blushing Akito' (B), 'Sweet' Akito' (C). Data are means of three replications, ± SD.

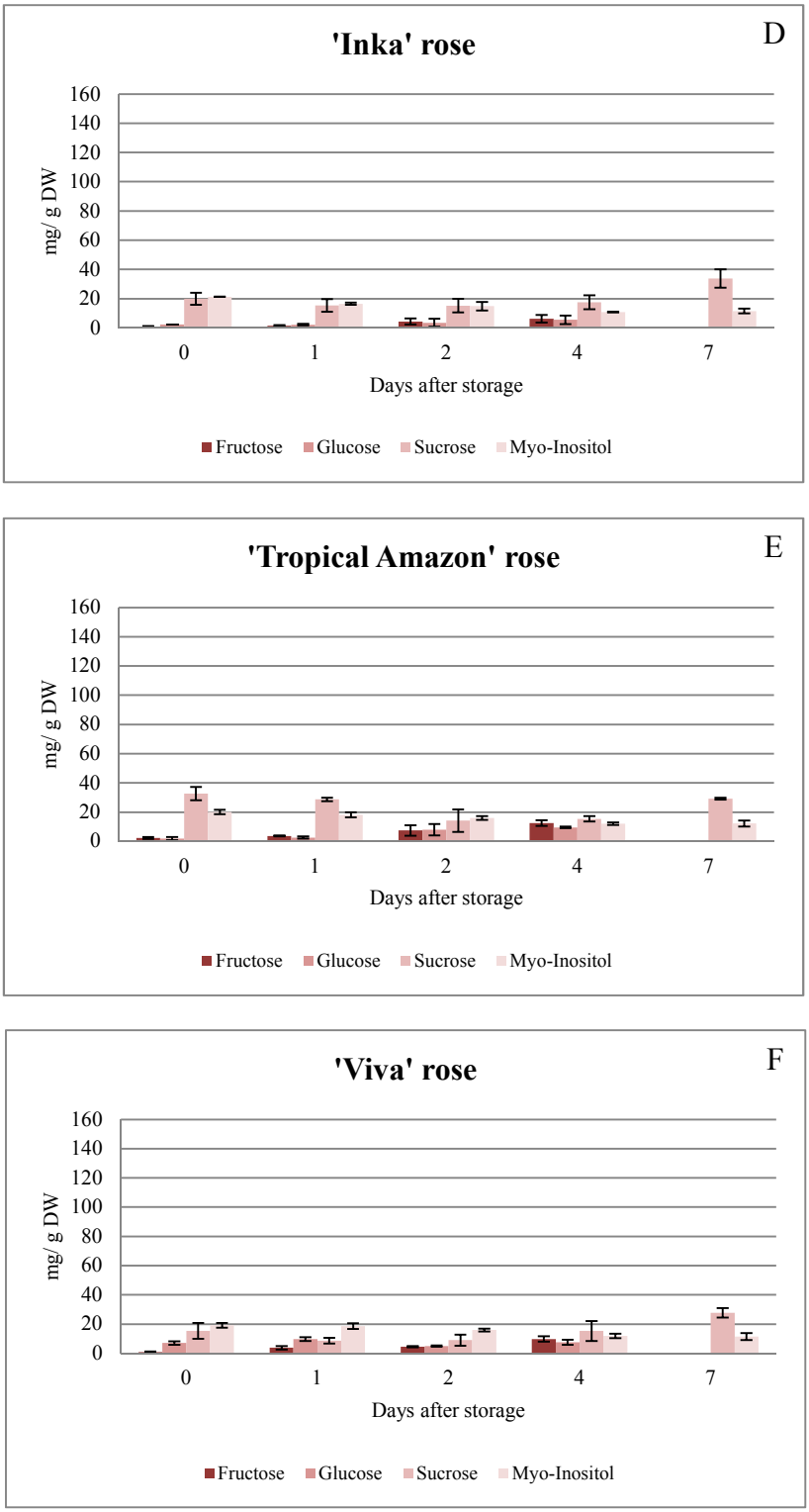


Figure 2.3 (Conc.): Changes in sugars in the foliage of nine cultivars of cut roses during seven days of vase life, 'Inka' (D), 'Tropical Amazon' (E), 'Viva' (F). Data are means of three replications, ± SD.

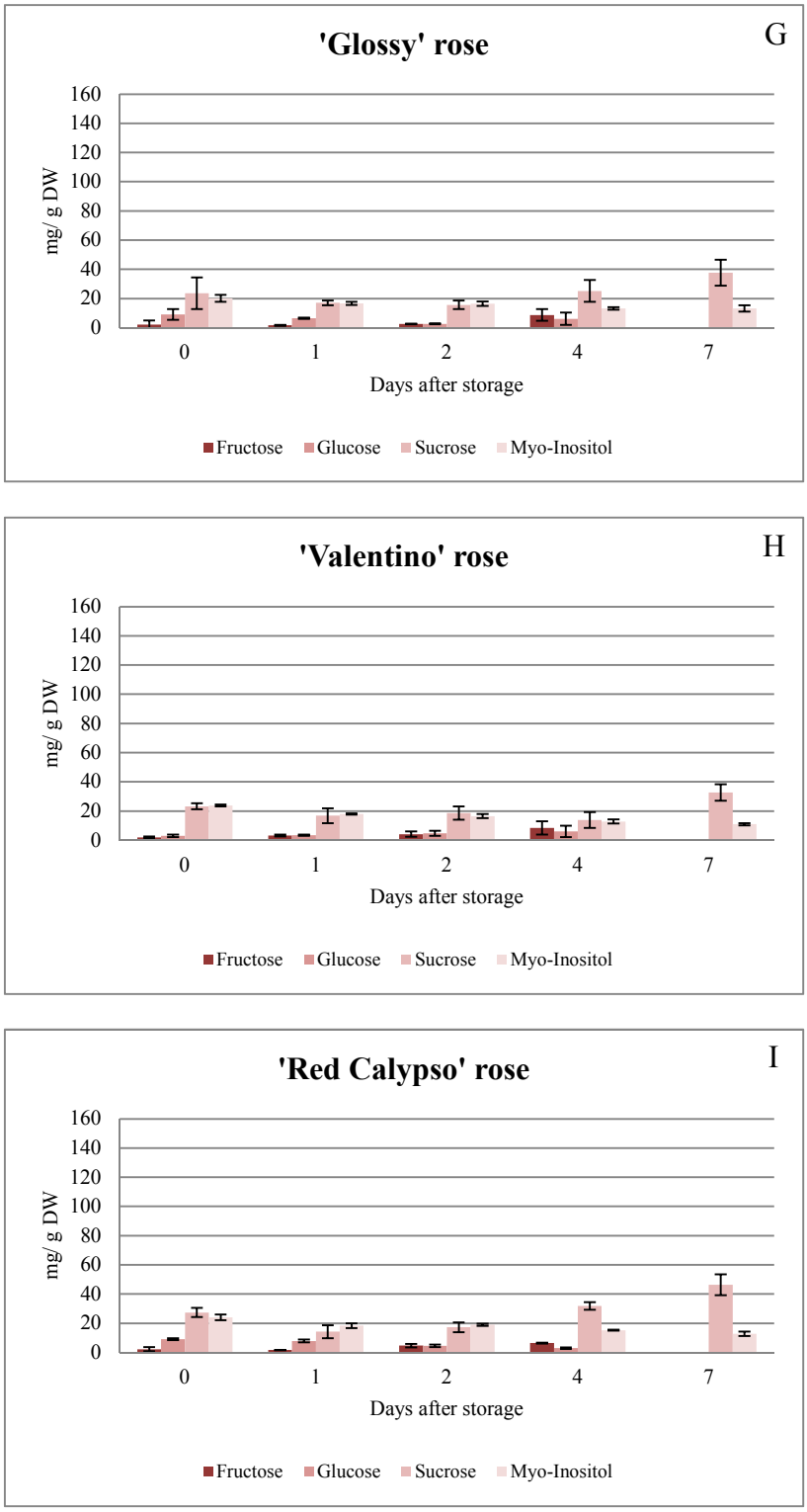


Figure 2.3 (Conc.): Changes in sugars in the foliage of nine cultivars of cut roses during seven days of vase life, 'Glossy' (G), 'Valentino' (H), 'Red Calypso' (I). Data are means of three replications, ± SD.

Table 2.2: Changes of total sugars content in the petals of nine cultivars of cut roses during seven days.

Cultivars	Total sugars content (mg/g DW)				
	Days				
	0	1	2	4	7
Akito	211.4^{a}	144.7^{bc}	177.8^{a}	157.2^{a}	113.1^{a}
Blushing Akito	147.4^{cd}	134.5^{bc}	150.0^{bc}	120.3^{bc}	123.5^{a}
Sweet Akito	190.5^{ab}	141.8^{bc}	175.1^{a}	132.0^{b}	122.8^{a}
Inka	100.2^{e}	109.3^{cd}	99.5^{d}	84.9^{d}	101.9^{a}
Tropical Amazon	152.5^{c}	201.5^{a}	174.7^{a}	123.6^{bc}	107.9^{a}
Viva	118.0^{de}	158.0^{b}	132.7^{c}	113.8^{bc}	116.0^{a}
Glossy	48.1^{f}	84.7^{d}	73.8^{e}	54.7^{e}	53.9^{b}
Valentino	166.4^{bc}	111.0^{cd}	134.9^{c}	99.8^{cd}	98.7^{a}
Red Calypso	151.9^{c}	157.9^{b}	163.7^{ab}	122.4^{bc}	132.3^{a}

Means of the same main effect within a column followed by the same letter(s) are not significantly different at P = 0.001 probability level.

Table 2.3: Changes of total sugars content in the foliage of nine cultivars of cut roses during seven days.

Cultivars	Total sugars content (mg/g DW)				
	Days				
	0	1	2	4	7
Akito	46.6^{bcd}	46.9^{b}	46.4^{b}	43.3^{b}	34.3^{cd}
Blushing Akito	144.1^{a}	92.6^{a}	95.0^{a}	102.7^{a}	61.1^{a}
Sweet Akito	43.5^{cd}	49.3^{b}	47.2^{b}	34.3^{b}	32.7^{d}
Inka	47.2b^{cd}	40.3^{b}	39.6^{b}	40.5^{b}	45.3^{abcd}
Tropical Amazon	58.6^{bc}	54.9^{b}	42.7^{b}	50.1^{b}	41.5^{cd}
Viva	40.7^{d}	38.6^{b}	36.3^{b}	45.4^{b}	39.2^{cd}
Glossy	53.7^{bcd}	40.2^{b}	36.9^{b}	50.4^{b}	50.9^{abc}
Valentino	53.7^{bcd}	43.4^{b}	44.4^{b}	39.9^{b}	43.6^{bcd}
Red Calypso	62.6^{b}	41.7^{b}	45.5^{b}	57.6^{b}	59.1^{ab}

Means of the same main effect within a column followed by the same letter(s) are not significantly different at P = 0.001 probability level.

Table 2.4: Correlation between vase lives of nine cultivars of cut roses and sugar content in petals of all cultivars at day 7.

Sugar	Correlation (R^2)
Fructose	0.0488
Glucose	0.0244
Sucrose	0.4211
Myo-inositol	0.1724
Total sugars	0.0119

2.3.2 Experiment II Overall appearance changes during vase life of two cultivars of cut lily and changes in sugar contents at different position

2.3.2.1 Variations in individual weight, time of opening, individual bud life and longevity of two cultivars of cut lilies

In this experiment, two varieties of 'Oriental' lily were investigated for overall appearance changes during vase life. The inflorescence longevity of the lily depends on the number of buds per inflorescence, their opening and individual bud life (van der Meulen-Muisers *et al*., 1999). This experiment selected an inflorescence that consists of 4 buds. A senescence of the primary and secondary bud is 50% of the whole inflorescence. Individual bud life of the primary and the secondary bud has an important effect on evaluation of longevity. Therefore, this experiment was intended to focus on changes that occur in both buds. Moreover, the time to opening and vase life of individual buds was evaluated for 'Mother's Choice' (White) and 'Tiber' (Pink) lilies.

The weights of the primary and secondary bud of inflorescences were measured. Figure 2.4 shows changes in weight of the individual buds of two cultivars. The weight of the primary and secondary bud of 'Mother's Choice' and

'Tiber' lily started to increase after day 0 until day 4, then the weight started to decline (Figure 2.4).

The time of opening of individual buds was observed (Figure 2.5). The primary and secondary buds of 'Mother's Choice' opened at days 8.5 and 9.5 while the first and second buds of 'Tiber' opened at days 5.5 and 7.0.

In addition, there were differences in inflorescence longevity among varieties. Inflorescence longevity of each variety was evaluated by flower quality. Stage 5 was used as the criteria for indicating the end of inflorescence life. Figure 2.6 shows changes of flower quality in 'Mother's Choice' and 'Oriental' lilies. 'Tiber' lilies reached stage 5 before 'Mother's Choice'. However, longevity depends on individual bud life in the same inflorescence. Individual bud lives of the primary and the secondary bud from the bottom were observed. Individual bud lives of the primary and secondary buds of 'Mother's Choice' were 14.0 and 15.3 days, respectively. While individual bud lives of the primary and secondary buds of 'Tiber' were 10.1 and 10.5 days, respectively (Figure 2.7). Inflorescence longevities of 'Mother's Choice' and 'Tiber' overall were 15.4 and 10.4 days (Figure 2.8).

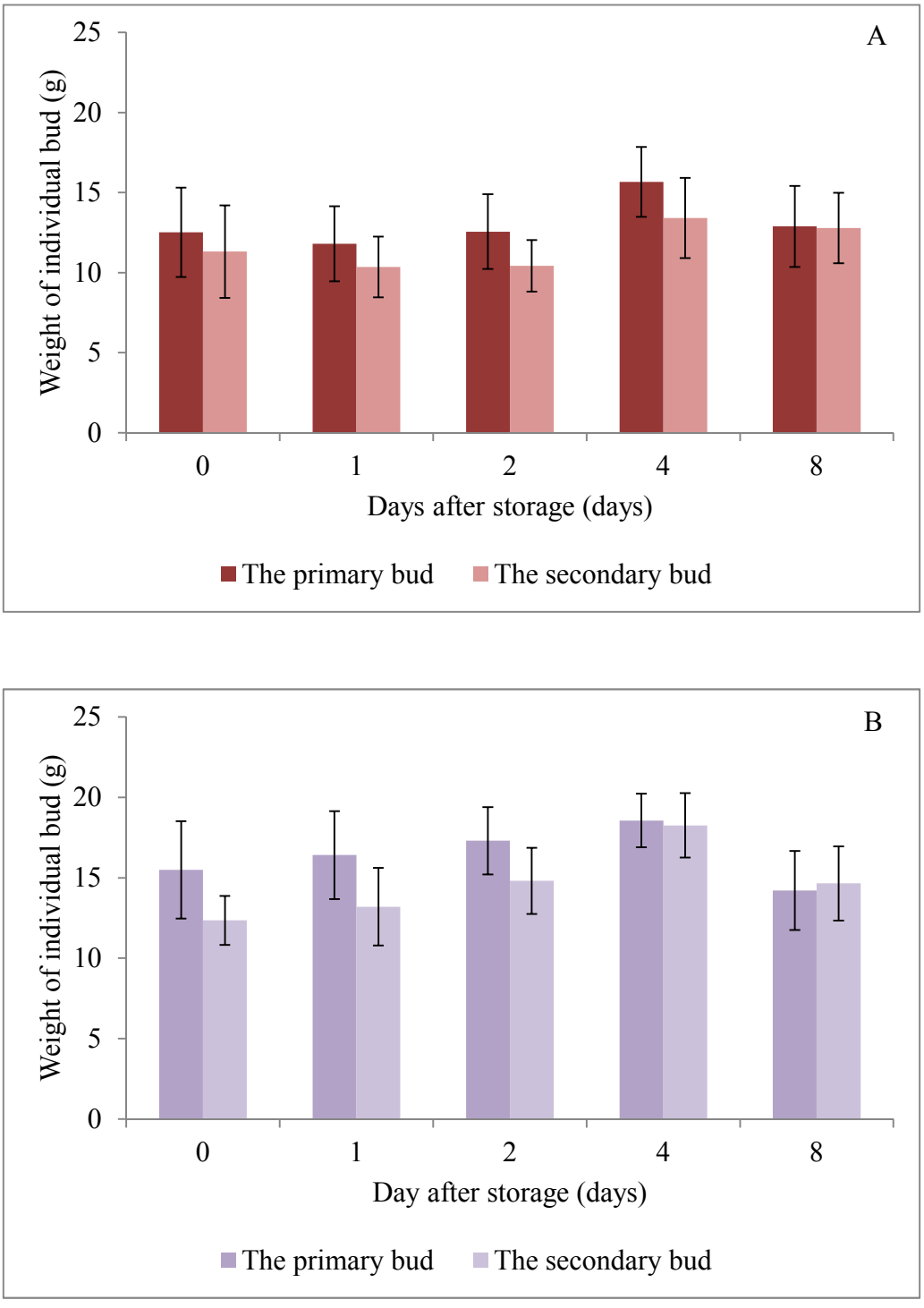


Figure 2.4: Change in weight of individual buds of lily flower, 'Mother's Choice' (A), 'Tiber' (B). Data are means of three replications, ± SD.

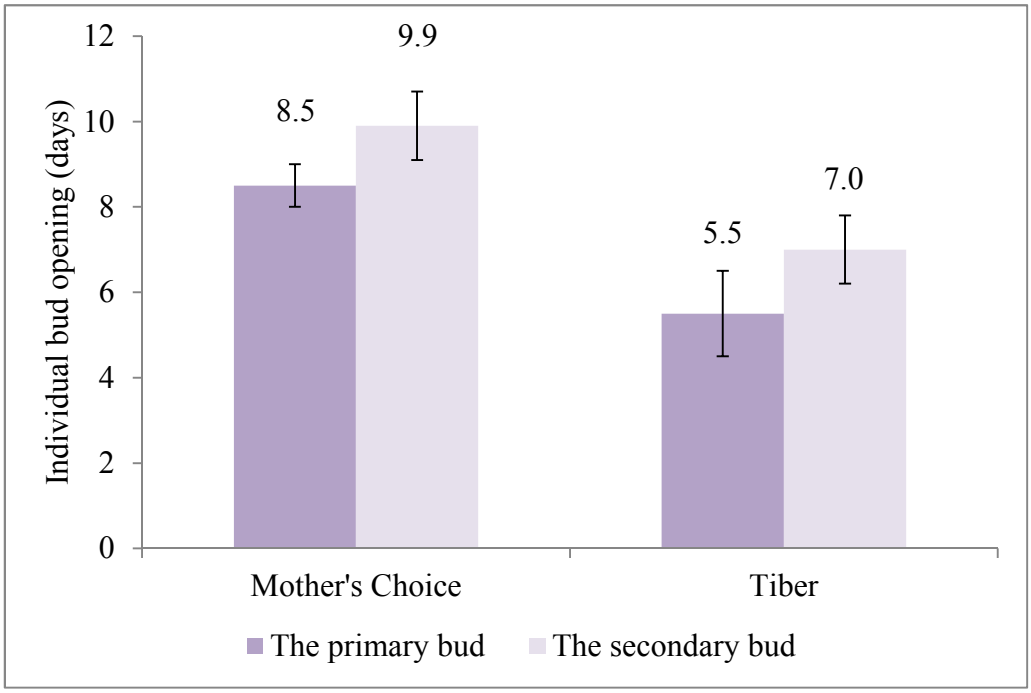


Figure 2.5: Individual bud opening of cut lily flowers. Data are means of three replications, ± SD.

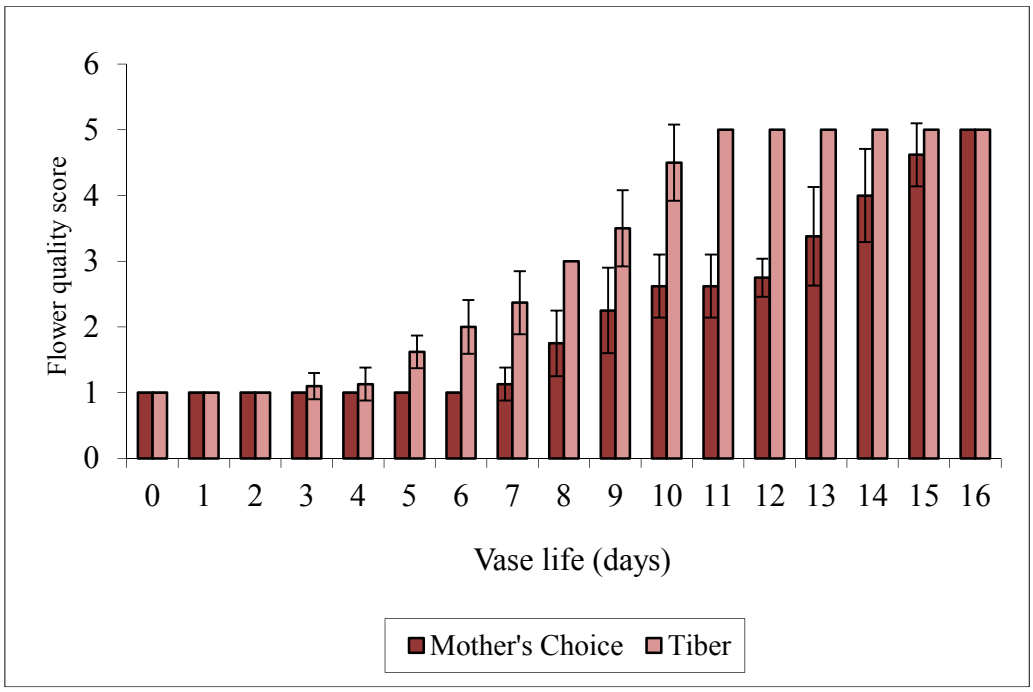


Figure 2.6: Changes in flower quality of cut lily over vase life. Data are means of three replications, ± SD.

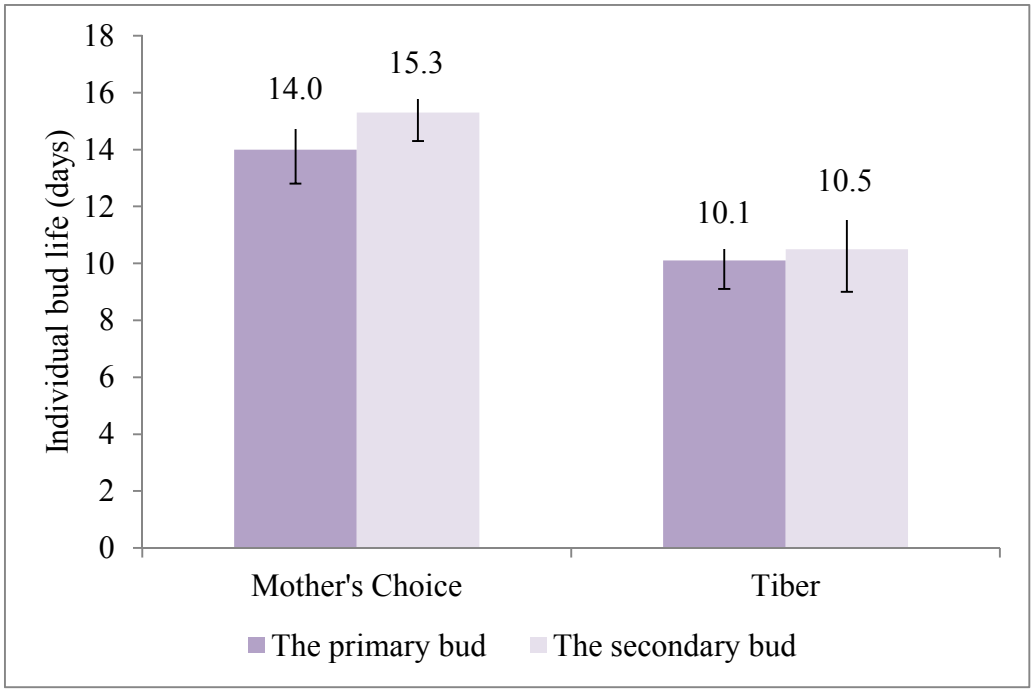


Figure 2.7: Individual bud life of cut lily flowers. Data are means of three replications, ± SD.

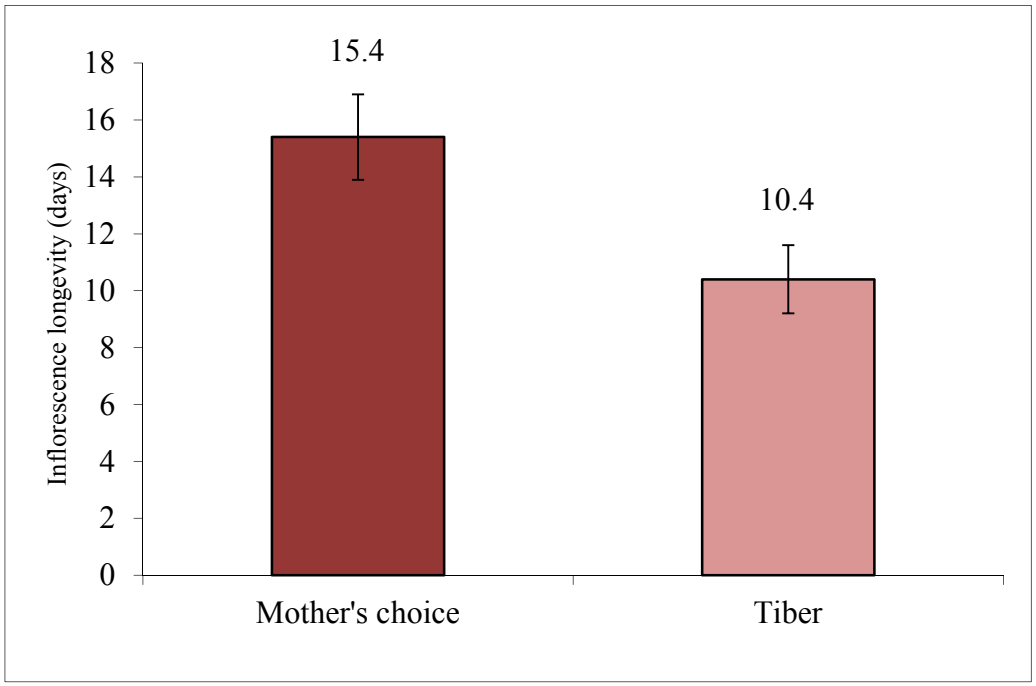


Figure 2.8: Inflorescence longevity of cut lily flowers. Data are means of three replications, ± SD.

2.3.2.2 Changes of sugar contents of 'Tiber' at different positions

The two varieties of cut lily ('Mother's Choice', and 'Tiber') varied in vase life and individual flower life. Due to consumer expectations, vase life of any cut flowers should not less than seven days. In this experiment, changes in sugar content (fructose, glucose and sucrose) in the sepals of 'Tiber' lily were measured over eight days of vase life. The concentration of fructose, glucose, and sucrose in the primary bud ranged between 102.6-51.0 mg g^{-1} DW, 85.3-26.2 mg g^{-1} DW, and 40.24-10.91 mg g^{-1} DW respectively. For the secondary bud, the concentration of fructose, glucose, and sucrose ranged between 71.3-51.5 mg g^{-1} DW, 57.7-33.3 mg g^{-1} DW, and 31.75-8.59 mg g^{-1} DW respectively.

In the primary bud, fructose and glucose contents slightly declined during the eight days while in the secondary bud both the fructose and glucose was more constant and the contents of both sugars were higher than in the primary bud (Figures 2.9 and 2.10). Sucrose contents were constant in both the primary and secondary buds and appeared to increase after day 2 (Figure 2.11). For total sugars, the content of the primary bud slightly decreased during the eight days, the same as with the secondary bud. However, total sugars of the second bud increased again after day 4.

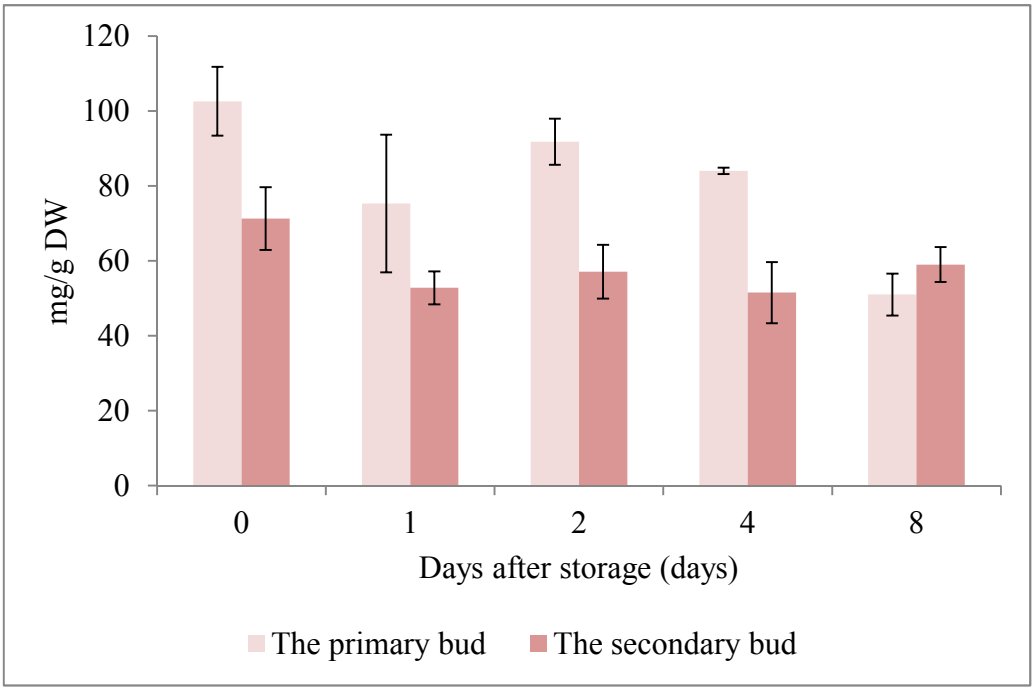


Figure 2.9: Change of fructose in sepals of 'Tiber' lily during eight days of storage. Data are means of three replications, ± SD.

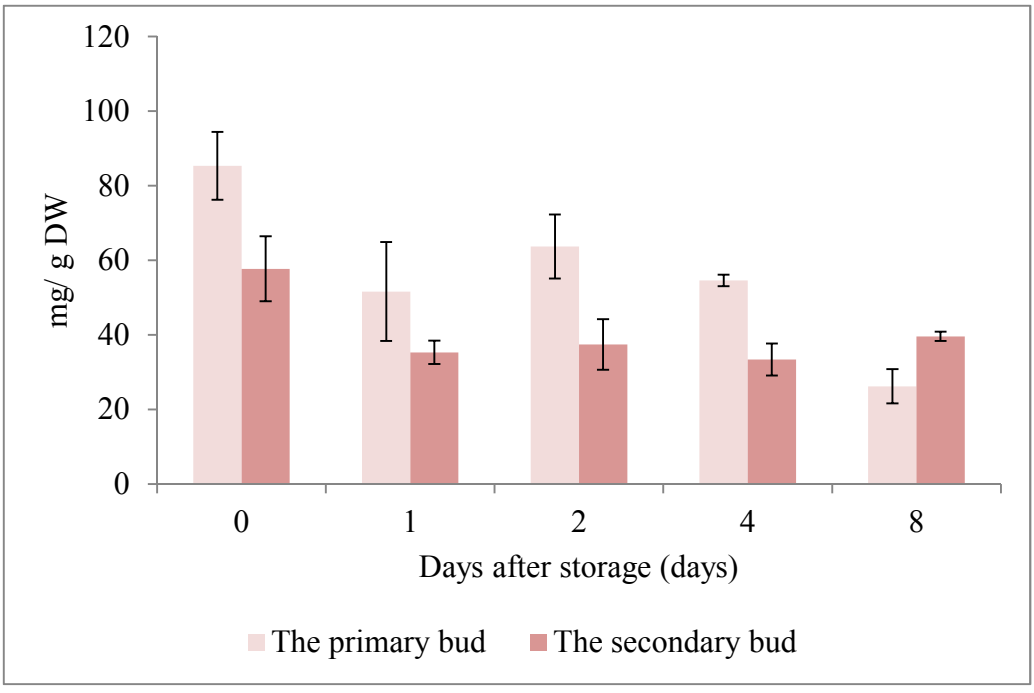


Figure 2.10: Change of glucose in sepals of 'Tiber' lily during eight days of storage. Data are means of three replications, ± SD.

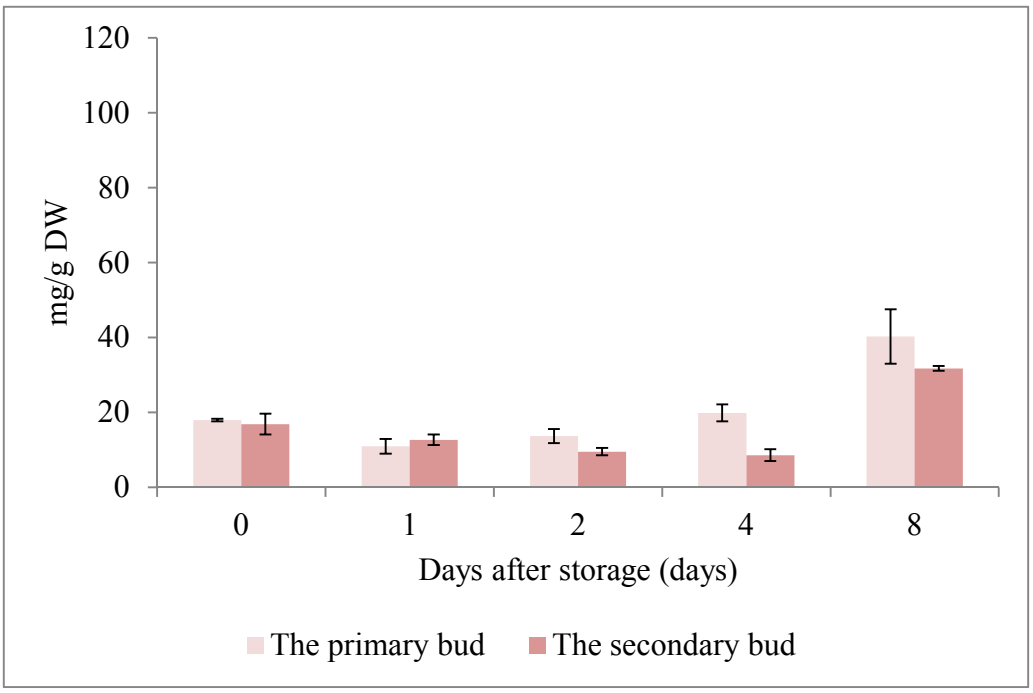


Figure 2.11: Change of sucrose in sepals of 'Tiber' lily during seven days of storage. Data are means of three replications, ± SD.

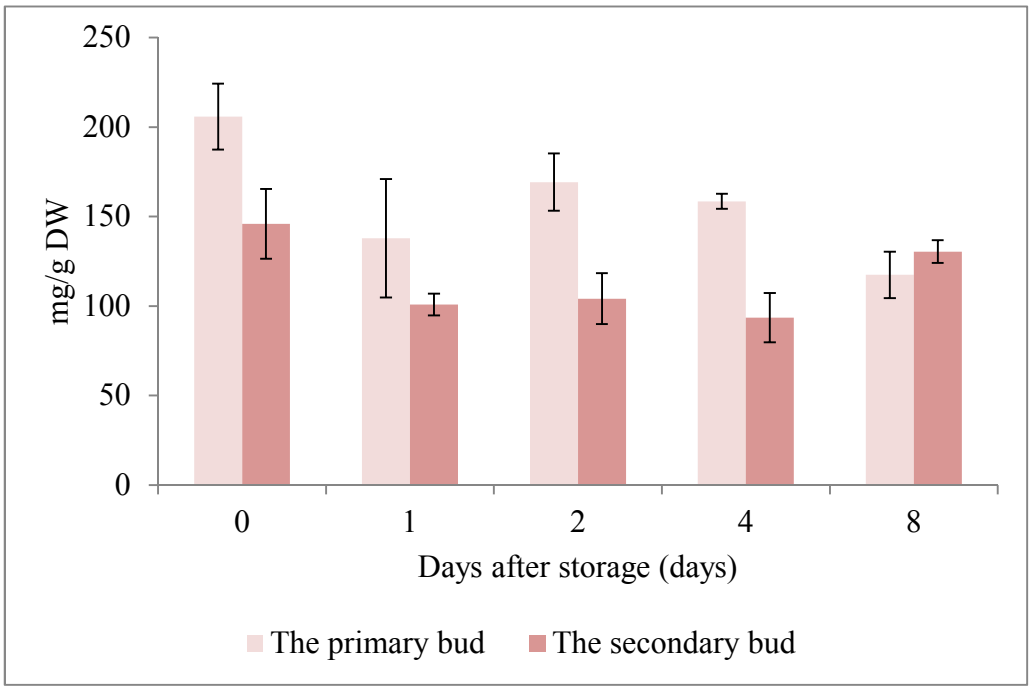


Figure 2.12: Change of total sugars in sepals of 'Tiber' lily during seven days of storage. Data are means of three replications, ± SD.

2.4 Discussion

2.4.1 Temporal changes in sugar content and overall appearance during the vase life of nine cultivars of cut rose

In the first experiment, cut roses as an example of a single flower that is used in mixed bouquets, were observed. Changes of overall appearance of nine cultivars of cut rose ('Akito', 'Blushing Akito', 'Sweet Akito', 'Glossy', 'Inka', 'Red Calypso', 'Tropical Amazon', 'Valentino' and 'Viva') were investigated throughout their vase life. Standard criteria of bud opening, flower quality and leaf quality were created.

For bud opening, changes from tight bud stage to fully open were observed. Most flowers did not completely open. Only 'Red Calypso' and 'Tropical Amazon' fully opened. During the vase life of roses, buds are expected to fully open before senescence. Marissen and Brijn (1995) report a difference in bud opening among various cultivars of cut roses, i.e. some cultivars have a good opening, some cultivars show a slower opening. However, roses often fail to open before senescence.

The flower quality of all cultivars reached stage 5 before the leaf quality did. This indicated that flower parts reach an unacceptable stage while leaf quality is still acceptable. Using flower quality standard criteria alone may be suitable for vase life assessment since the flowers are the most important components for consumers.

Vase life itself is a measure of longevity postharvest and is defined by the number of days from harvest to the senescence stage (Teixeira da Silva, 2003). Assessment of vase life shows that there are various lengths of vase life among the nine cultivars; the longest was 'Red Calypso' followed by 'Valentino', 'Glossy', 'Viva', 'Tropical Amazon', 'Inka', 'Sweet Akito', 'Blushing Akito' and 'Akito'. Differences in vase life may be influenced by genotype, environment pre-harvest, and postharvest management methods and conditions (Joyce and Faragher, 2012).

Vase lives of the nine cultivars varied from 4.5 to 11.5 days. Generally, consumers assume the vase life of cut flower should be not less than seven days (Ranwala, 2007). Therefore, the content of fructose, glucose, sucrose and myo-inositol was analyzed during the first seven days of vase life. This experiment found a difference in content of fructose, glucose, sucrose, and myo-inositol in petals and

leaves among the nine varieties of cut roses. However, levels of fructose, glucose, sucrose and myo-inositol in all cultivars had the same trend. The content of fructose and glucose found in petals was higher than in leaves. On the other hand, the content of sucrose and myo-inositol found in petals was less than in leaves.

Levels of fructose in petals of all cultivars seemed to be constant during seven days of vase life, while levels of glucose content slightly decreased. Vase lives of 'Akito', 'Blushing Akito' and 'Sweet Akito' were less than seven days. They have contents of fructose and glucose that are not different from other cultivars at day 7. However, it has been reported that fructose and glucose are the main sugars in petals (Ichimura *et al*., 1999). Sucrose transferred from other organs to petals, is reported to be metabolized into glucose and fructose and accumulates in petals (Yamada *et al*., 2007). Moreover, van Doorn (2001) reports that soluble sugars in petals are still high at the time of senescence; similarly, Kumar *et al*. (2008) suggest that sugar concentrations are still high when petals reach the early stages of senescence. Perhaps, therefore, it is not surprising that the levels of glucose and fructose remained relatively constant and static during these experiments, although a general flux of sugars including sucrose from leaves to petals may be expected, which was not generally evident.

However, the levels of fructose and glucose in leaves were less than in petals; the levels of fructose and glucose decreased and could not be detected at day 7. Decreasing of both fructose and glucose may relate to the increasing of sucrose content in leaves. In this experiment, an increase in sucrose content in the leaves of all cultivars at day 7 was found. A high level of sucrose found in leaves may be due to leaves being the source of carbohydrate supply for flower buds (Marissen and Brijn, 1995). In roses, leaves act as a storage pool for carbohydrate, which is transported to the flower bud during vase life (Marissen, 2001).

The level of myo-inositol in both petals and leaves was slightly decreased over seven days. These changes were the same as the findings of Foukaraki (2008) who reports cut 'First Red' roses showed a decrease in sucrose, glucose and myo-inositol concentration in their petals during vase life.

This experiment found values of correlation (R^2) of all the sugar contents in petals at day 7 with vase lives were not accepted. Therefore, this indicated that levels of each sugar content in petals may not relate to long-lived cultivars. However, Marissen (2001) suggests that accumulation of sugars may relate to other factors. Only the carbohydrate contents in the leaves and petals could not indicate of vase life.

2.4.2 Overall appearance changes during the vase life of two cultivars of cut lily and changes in sugar contents at different positions

The oriental lily was selected for study as a sample of an inflorescence-type of flower; two varieties of 'Oriental' lily were investigated for overall appearance changes during vase life. Weight of individual buds, the time to opening and longevity of individual buds were evaluated for 'Mother's Choice' (White) and 'Tiber' (Pink) lilies.

For cut lilies, assessment of longevity life always focuses on individual bud life in the same inflorescence. Investigation into changes of flower quality and longevity life of 'Mother's Choice' and 'Tiber' lilies found the same pattern of growth and development in the primary and the secondary bud even though their longevity lives were different.

Differences in developing bud stages at harvest affect individual flower longevity (van der Meulen-Muisers *et al*., 1998). Growth and development in the primary bud always occur before the secondary bud. Although the primary buds gained more fresh weight than the secondary buds, finally their fresh weights were not different after they started to open. Moreover, individual bud opening and individual bud life of the primary and secondary buds were not different in either variety. In this experiment, cut oriental lilies with a stem that consisted of four buds were selected. The results showed that changes of overall appearance occurring in the primary and secondary bud were more than for the other buds. When the primary and secondary bud reached an unacceptable stage, the percentage of senescence of the whole inflorescence is around 50 to 60%. Therefore, the individual bud life of the primary and secondary bud may indicate the longevity life of lilies. Van der Meulen-Muisers *et al*. (2001) suggest that the vase life of lily flowers depends on the number of buds

within the inflorescence after harvest. In 1998, van der Meulen-Muisers *et al*. reported that different genotypes show variations in the individual flower life of cut lilies.

The level of sugars in the primary and secondary buds of 'Tiber' lilies were measured during first eight days of storage life. In the primary bud, the content of fructose and glucose was slightly decreased while the content of fructose and glucose in the secondary buds was constant. This experiment found a contrast in sucrose content in the primary and secondary buds even though the level of sucrose was lower than fructose and glucose. The content of sucrose slightly decreased during first stage then the level of sucrose increased again before bud opening. In the primary bud, sucrose content increased at day 4 and the bud started to open around days 5 to 6. For the secondary bud, sucrose content increased after day 4 and the bud started to open around day 7. An increase in total sugars in the primary and secondary bud was also a result of increasing sucrose content. The results found in this experiment were the same as those of Arrom and Munne-Bosch (2012) who report that endogenous glucose contents decrease during senescence in all floral organs, while sucrose contents increase in the outer and inner sepals.

In the case of 'Tiber' lily, the contents of fructose, glucose, sucrose were measured during first eight days of storage and longevity life was longer than 10.4 days. Moreover, the individual bud lives of the primary and secondary buds were not different. Therefore differences in levels of fructose, glucose and sucrose in the primary and secondary bud but may not relate to the longevity of individual bud life. An increase in sucrose content may be related to a role in resource of bud opening. Van der Meulen-Muisers *et al*. (2001) found an increase of sucrose content in the sepals with bud development, but it remained at relatively low levels for all stages.

2.5 Conclusions

This experiment studied the overall appearance changes of single-type and inflorescence-type flowers. Cut roses were selected as an example of a single-type flower and oriental lilies as an example of an inflorescence-type. Overall appearance changes were described using standard criteria for the evaluation of flower quality, leaf quality and bud opening, which were specifically developed for this study.

In cut rose flowers, the vase lives of nine cultivars (‘Akito’, ‘Blushing Akito’, ‘Sweet Akito’, ‘Glossy’, ‘Inka’, ‘Red Calypso’, ‘Tropical Amazon’, ‘Valentino’ and ‘Viva’) were assessed by using flower quality. There was a difference in vase life among cultivars. Sugars in petals and leaves were measured for their contents in relation to short-lived and long-lived cultivars. There were varying contents of fructose, glucose, sucrose and myo-inositol in all cultivars. However, levels of all sugars were not related to short-lived and long-lived cultivars.

For cut lily flowers, the overall appearance changes during vase life were investigated in ‘Mother’s Choice’ and ‘Tiber’ lilies, although the longevity life of ‘Mother’s Choice’ lilies was longer than ‘Tiber’ lilies. Due to the difference in the individual bud life of the primary and secondary bud of both cultivars were not significantly different, therefore, the individual bud life of the primary and secondary bud may therefore be suitable for the evaluation of inflorescence longevity.

Moreover, the contents of fructose, glucose and sucrose were measured in the primary and secondary bud of ‘Tiber’ lilies. Differences in the content of fructose glucose and sucrose between the primary and secondary bud did not relate to the longevity of individual bud life. However, this experiment found increases in sucrose contents in both positions before the time of opening. This increase in sucrose may relate to the role of the carbohydrate energy source for bud opening.

CHAPTER THREE

Role of bacterial populations in vase water on the vase life of mixed flower bouquets

3.1 Introduction

An increase of bacteria in vase solutions and cut stems could cause xylem occlusion by physical blockage from their cells and products, stimulating emboli in the xylem, causing cellular malfunction through toxic metabolite production and enzyme action with degraded cell walls, and/or by endogenous ethylene production (Ratnayake *et al*., 2012). The vase life of cut rose flowers is often reduced because of water stress symptoms such as wilting and bent neck, which may be caused by blockage. Such blockage may relate to an increase in the number of bacteria at the cut surface and inside the stems (van Doorn et al., 1991). The role of microorganisms in the vase life of cut rose flowers has been studied by the inclusion of antimicrobial compounds or bacteria in the vase solution. These studies show that the onset of water stress symptoms is delayed by including antimicrobial compounds and is advanced by including bacteria in the vase water (Van Doorn and de Witte, 1991).

Several bacteria and fungi, which had been isolated from cut flower stems and vase water, have been studied in many research projects, such as those concerning the chrysanthemum, gerbera and rose. Generally bacteria present on the stems were the same as in vase water. *Enterobacter*, *Bacillus spp*. and fungi were present at the early stages in vase water and then lost their dominance, being replaced by *Pseudomonas spp.* (Put, 1990).

However, the previous research has been conducted on a single flower type while mixed flowers bouquets have not been studied. It may be that the bacterial populations found in mixed bouquets are different in term of size or structure due to the numbers of stems used and the combinations of varieties that are brought together. It is not known whether simply adding additional stems has a significant effect on bacterial populations and vase life, and the changes imposed by mixing together varieties has not been studied up until now. The objective of this experiment was to

study the role of different numbers of stems on bacterial population and the vase life of cut flowers. The results of this experiment may provide initial information for further studies on the role of microorganisms on the vase life of mixed flower bouquets.

3.2 Materials and methods

3.2.1 Plant material

'Akito' and 'Valentino' roses and 'Tiber' lilies were obtained from Flamingo Holdings Company *Ltd*., (Gt North Road, Sandy, Bedfordshire SG19 2AJ). Then all the flowers were transported to the Microbiology Laboratory at Cranfield University, UK. After that, they were selected for uniformity and defective stems were rejected. The stems of all samples were re-cut into 50 cm lengths under water using a sterile razor blade (van Doorn, 1997). Leaves on the lower one-third of stems were stripped. Within each stem of the lilies, the number of buds per stem was kept as constant as possible for determining the stem vase life.

3.2.2 Experiment design

Experiment 3.1: cut 'Tiber' lilies were stood in a 2-Litre vase containing 1,000 ml distilled water with single and two stems.

Experiment 3.2: cut 'Akito' roses were stood in a 2-Litre vase containing 1,000 ml distilled water with single and five stems.

Experiment 3.3: cut 'Valentino' roses were stood in a 2-Litre vase containing 1,000 ml distilled water with single and five stems.

Experiment 3.4: mixed flowers were stood in a 2-Litre vase containing 1,000 ml distilled water. There were two treatments; mixture of a single stem of 'Akito' rose with a 'Tiber' lily (1:1 treatment) and a mixture of five stems of 'Akito' rose with two stems of 'Tiber' lilies (2:5 treatment).

Experiment 3.5: mixed flowers were stood in a 2-Litre vase containing 1,000 ml a commercial liquid flower food for roses. There were two treatments; mixture of a single stem of 'Akito' rose with a 'Tiber' lily and mixture of five stems of 'Akito'

rose with two stems of 'Tiber' lilies (the latter combination is the one that is usually commercially available).

Experiment 3.6: bacterial numbers were evaluated on cut surface, outer stem and in xylem of 'Akito' roses and 'Tiber' lilies.

The experiment was a completely randomised design (CRD) with six replications. A CRD was adopted in the vase life room. The vases of flowers were placed in the vase life room at 20°C and given a 12-hour on-off light cycle under a lamp throughout the duration of the experiments.

3.2.3 Preparation of samples for microorganism estimation

Vase water was sampled after stirring using an aseptic 3 ml pipette which discharged the sample into a sterile bottle. There were three replications per treatment and each replication was collected from two vases (There were six vases per treatment). Samples from the vases were taken aseptically each third day of the vase life until flowering senescence. Protection against contamination was achieved by sealing a plastic film to the top of each vase.

3.2.3.1 Isolation and identification of microorganisms

Bacteria were isolated from vase water were collected from cut roses and lilies. One mL of vase water was serially diluted into 10 to 1,000-fold dilutions, and 100 µL of vase water were spread on sterile Tryptone Soya Agar (Oxoid), MacConkey agar (Oxoid), *Pseudomonas* selective agar (Oxoid), and Columbia CNA (Oxoid). All agars were sterile by using steam autoclave at 121°C with 15psi pressure for 15min. Total bacterial plate counts were evaluated on Tryptone Soya Agar (TSA). Gram negative bacteria were investigated on MacConkey agar and *Pseudomonas* selective agar. Columbia CNA evaluated Gram positive bacteria. The plates were incubated for 48 hr at 25°C, and individual colonies of microorganisms, that emerged during periods of time were isolated, then were purified by using a sterile loop and streaked on TSA. Purified bacteria were identified by using the results of the Gram stain and biochemical test; oxidase test (oxidase test disc, 70439 Oxidase Test, Fluka); and catalase test (3% hydrogen peroxide, Fluka).

Bacteria isolated from each experiment were each identified by a number and suffix; L stands for 'Tiber' lily, A stands for 'Akito' rose, V stands for 'Valentino' roses, LA stands for mixture of 'Tiber' lilies with 'Akito' roses held in distilled water, LAF stand for mixture of 'Tiber' lilies with 'Akito' roses held in flower food.

3.2.4 Physiological measurements

3.2.4.1 Vase life

The cut flower longevity was recorded as days of vase life from the time the flowers were placed into the vases (day 0). The end of the vase life was indicated when a score of flower quality reached stage 5 (refer to flower standard criteria in chapter 2).

3.2.4.2 Fresh weight and water uptake measurement

The stem fresh weight (f.w.) and the vase weight (vase + water) were measured at the same time every day from day 0. These data were used to determine the relative fresh weight (RFW) as a percentage of the initial fresh weight (% initial f.w.) and water usage was calculated as g/ initial f.w. per day.

3.2.4.3 Bacteria count on cut surface, outer stem and in the xylem

For determination of number of bacteria on or in the stem, stems were recut every 5 cm over a 50 cm length of 'Akito' rose stem and over a 30 cm length of 'Tiber' lily stem with a sterile blade. Sterile cotton buds were swabbed from the cut surface and outer stem (separate treatments) onto TSA. Swabs were taken from the cut surface to get an estimation of the bacteria 'released' from the tissues (including the xylem) by cutting. For determination of bacteria in xylem, a piece of stem (5 cm length) was cleaned in 50 ppm DICA (Sodium dichloroisocyanuric acid) for 5 min, then the stem was ground with 5 mL sterile distilled water using sterile mortar. 100 µL of vase water were spread on sterile TSA. The plates were incubated for 48 h. at 25°C before counting. Bacteria count was detected on day before (day 0) and being in vase at day 6 and day 12.

3.2.5 Statistical analysis

Significance tests by analysis of variance (ANOVA) using SPSS version 16 were applied to water uptake, relative fresh weight, total bacterial plate count and vase life. Mean comparisons were made using least significance difference (LSD).

3.3 Results

3.3.1 Bacteria populations in vase water and the vase life of the cut 'Tiber' lily

3.3.4.1 Changes of bacterial population in vase water of 'Tiber' lily

The total bacterial plate count in the vase water of the single and two stems of 'Tiber' lily are shown in figure 3.1. In the vase water of the single stem and two stems, the trend of bacteria population was constant during the first six days before slightly increasing after that. Then bacteria populations of both the single and two stems were not significantly different except for day 3 (Figure 3.1).

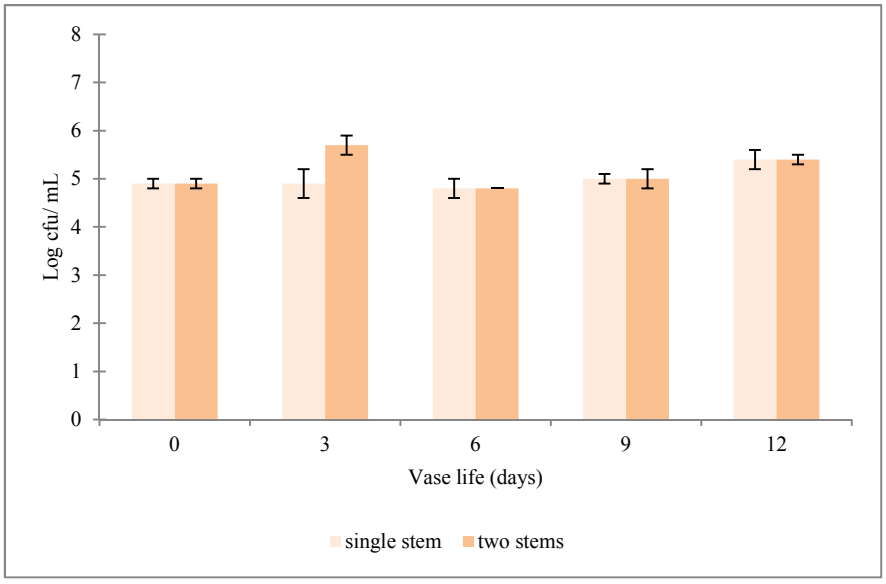


Figure 3.1: Changes of total bacterial plate count of cut 'Tiber' lilies during 12 days of vase life. Data are means of three replications, ± SD.

The vase water was studied for both the number of bacteria and the bacterial groups present. There were seven unique bacteria found in the vase water of 'Tiber' lilies (Table 3.1).

Table 3.1: Unique bacteria found in vase water of 'Tiber' lilies.

Species	**No.**
Staphylococcus spp.	L7
	L8
Streptococcus spp. or *Enterococcus spp.*	L9
Enterobacteria spp.	L4
Pseudomonas spp.	L15
	L29
Brucella spp.	L10

For *Staphylococcus spp.*, the number of L7 of both single and two stems were constant for 12 days except day 12 when the number of L7 in the vase water of the single stems was more than for the two stems. However, the number of L7 in the vase water of the single and two stems were not significantly different. Furthermore, the numbers of L8 in the vase water of the single and two stems were constant during vase life. The numbers of L8 in the vase water of the single stem and two stems were not significantly different (Figure 3.2).

L9 was bacteria in the group of *Streptococcus spp.* or *Enterococcus spp*. The number of L9 of both single and two stems decreased during first three days but increased again at day 6 before a slight decline throughout the vase life. However, the number of L9 in the vase water of the single and two stems was not significantly different (Figure 3.3).

Enterobacteria spp. (L4) of both the single and two stems showed an increase in number for the first time until day 6, then the numbers were constant until the end of vase life. A comparison between single and two stems showed that numbers of L4 were not significantly different except for day 3 (Figure 3.4).

Bacteria in the group of *Pseudomonas spp*. found in this experiment were designated as L15 and L29. Numbers of L15 were constant during vase life and there were no significant differences between single and two stems. Moreover, the number of L29 for single and two stems slightly increased over 12 days. However, data were not significantly different between single and two stems (Figure 3.5).

L10 was a bacterium in the group of *Brucella spp*. Numbers of L10 in the vase water of the single stem increased until day 6 then numbers were constant, while the numbers of L10 in the vase water of two stems were constant for 12 days. The vase water of the single stem had significantly more L10 than in the vase water of the two stems (Figure 3.6).

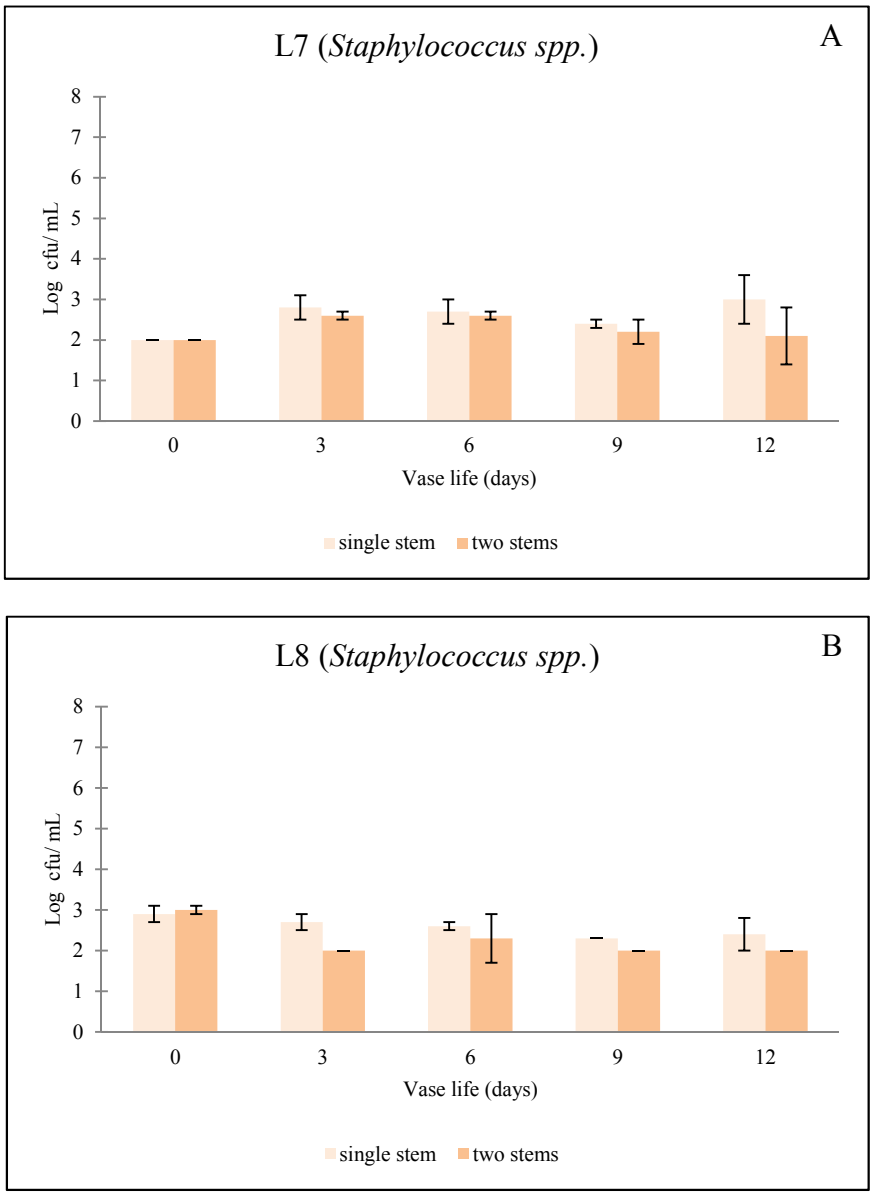


Figure 3.2: Changes of *Staphylococcus spp*. in vase water of cut lilies during 12 days of vase life: L7 (A), L8 (B). Data are means of three replications, ± SD.

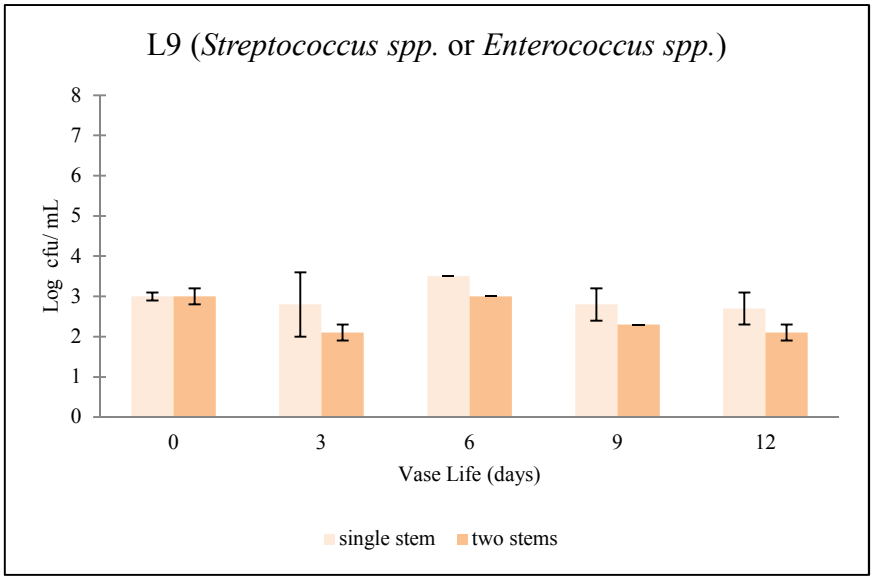


Figure 3.3: Changes of *Streptococcus spp.* or *Enterococcus spp.* (L9) in the vase water of cut lilies during 12 days of vase life. Data are means of three replications, ± SD.

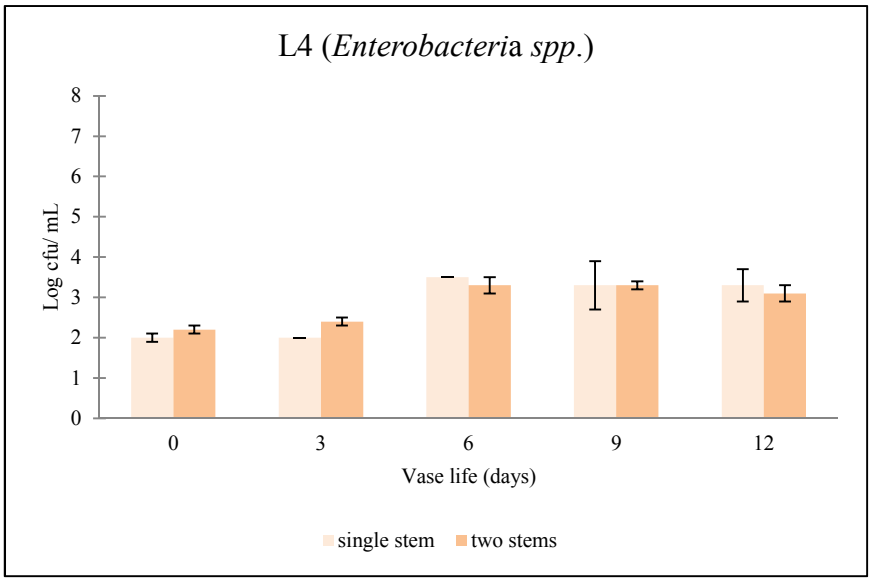


Figure 3.4: Changes of *Enterobacteria spp.* (L4) in the vase water of cut 'Tiber' lilies during 12 days of vase life. Data are means of three replications, ± SD.

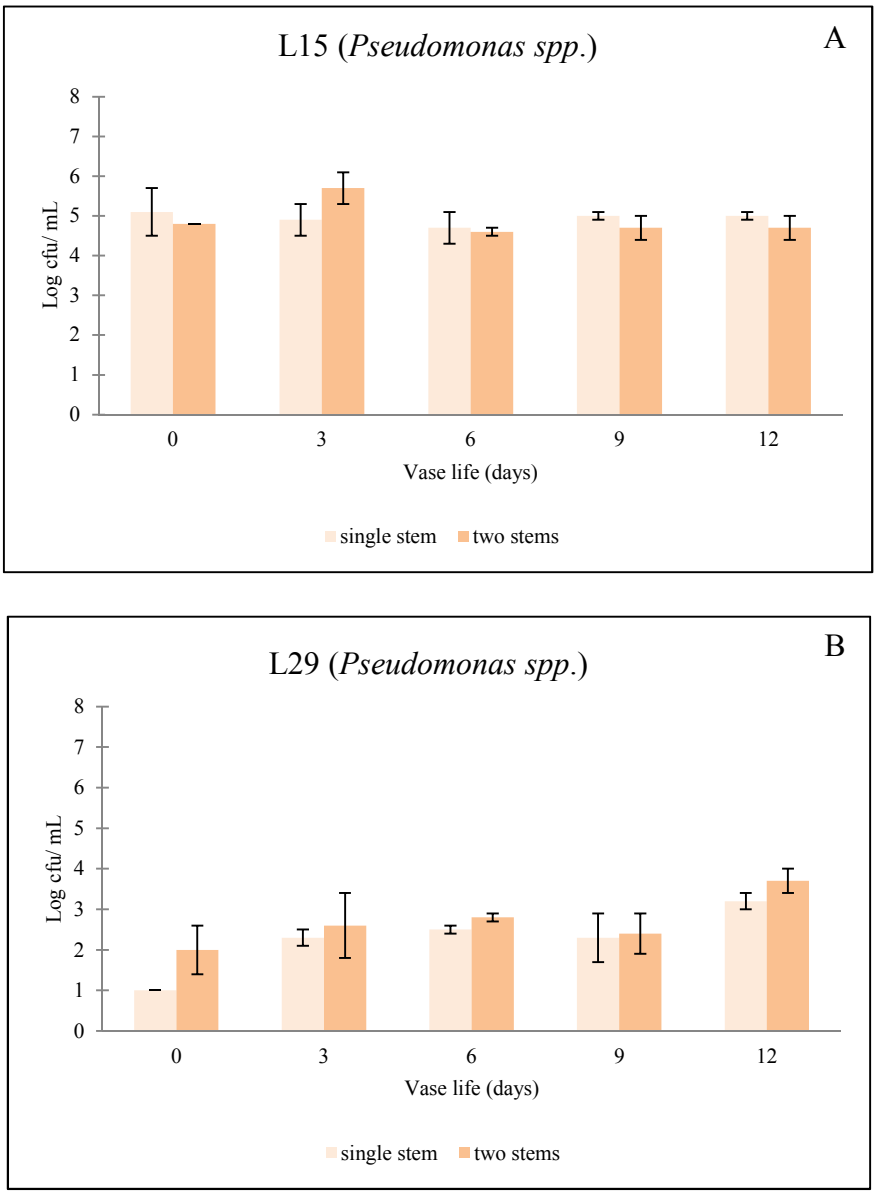


Figure 3.5: Changes of *Pseudomonas spp.* in vase water of cut 'Tiber' lilies during 12 days of vase life: L15 (A), L29 (B). Data are means of three replications, ± SD.

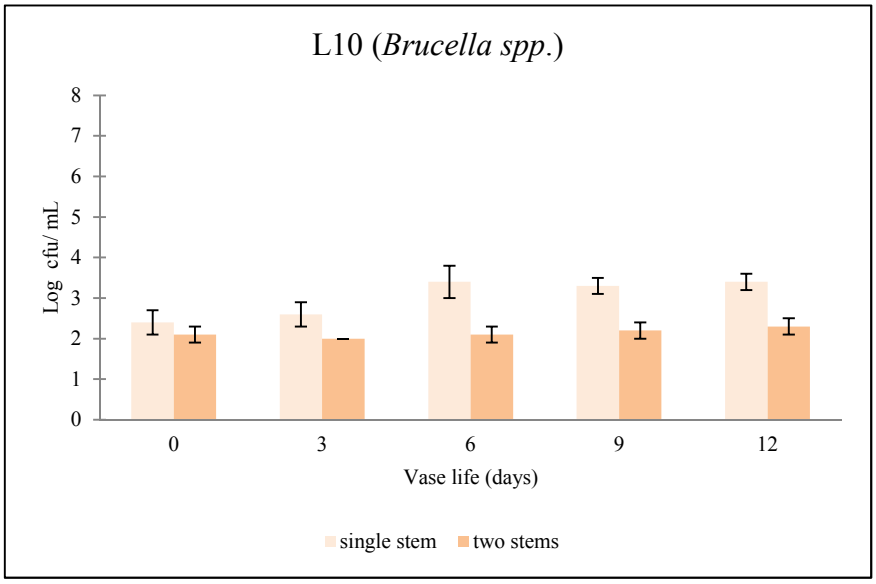


Figure 3.6: Changes of *Brucella spp*. (L10) in the vase water of cut 'Tiber' lilies during 12 days of vase life. Data are means of three replications, ± SD.

3.3.1.2 Changes of water uptake rate, fresh weight and vase life of the 'Tiber' lily

Changes of water uptake rate of the 'Tiber' lily were measured during vase life. The water uptake rate of a single stem increased until day 6 then was constant throughout the vase life while water uptake of the two stems slightly decreased after day 6. However, data for the water uptake of the single and two stems were not significantly different (figure 3.7).

Changes in fresh weight presented an increase in weight until day 6 before slightly declining during the vase life. However, the data of fresh weight of the single and two stems were not significantly different (Figure 3.8). Also, the vase lives of 'Tiber' lilies were not significantly different. The vase life of 'Tiber' lilies as a single stem was 11.5 days while for two stems was 11.1 days (Figure 3.9).

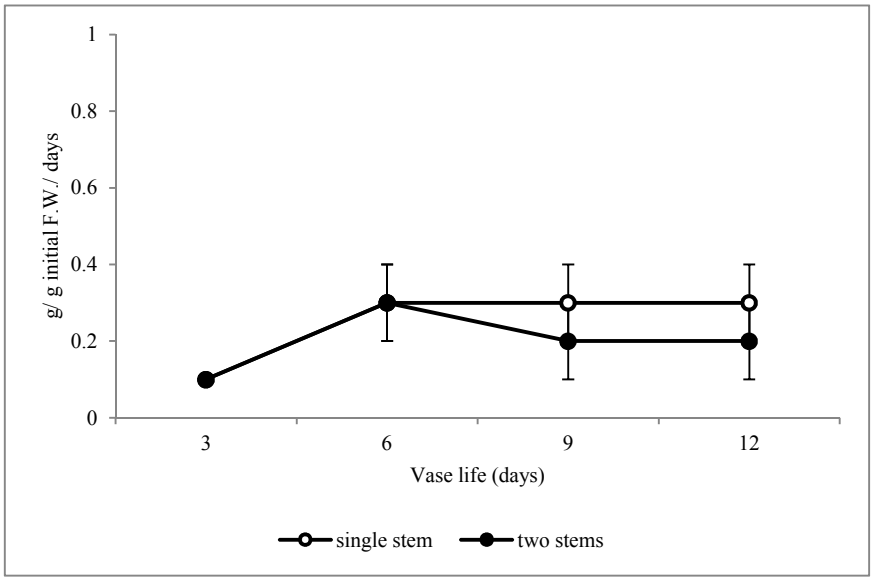


Figure 3.7: Changes of water uptake of cut for 'Tiber' lilies during the 12 days of vase life.

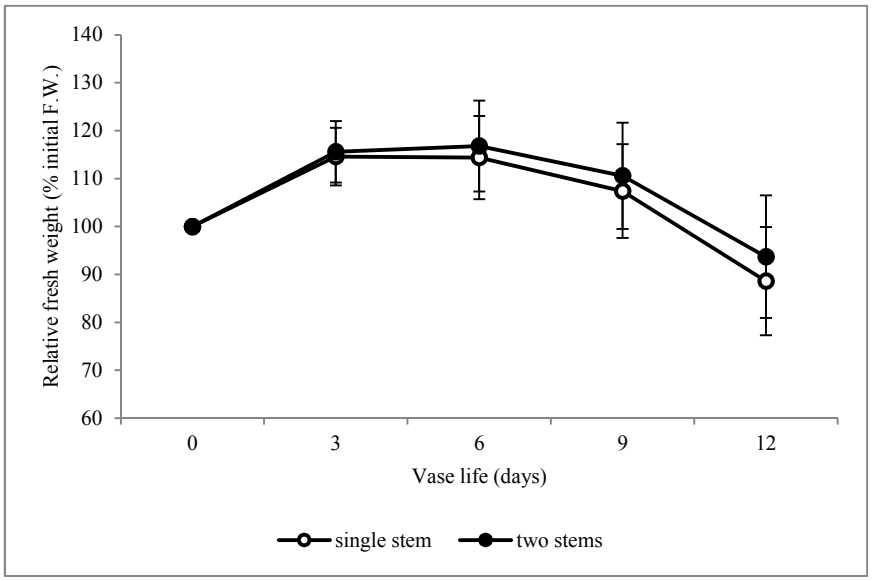


Figure 3.8: Changes of fresh weight of cut 'Tiber' lilies during the 12 days of vase life.

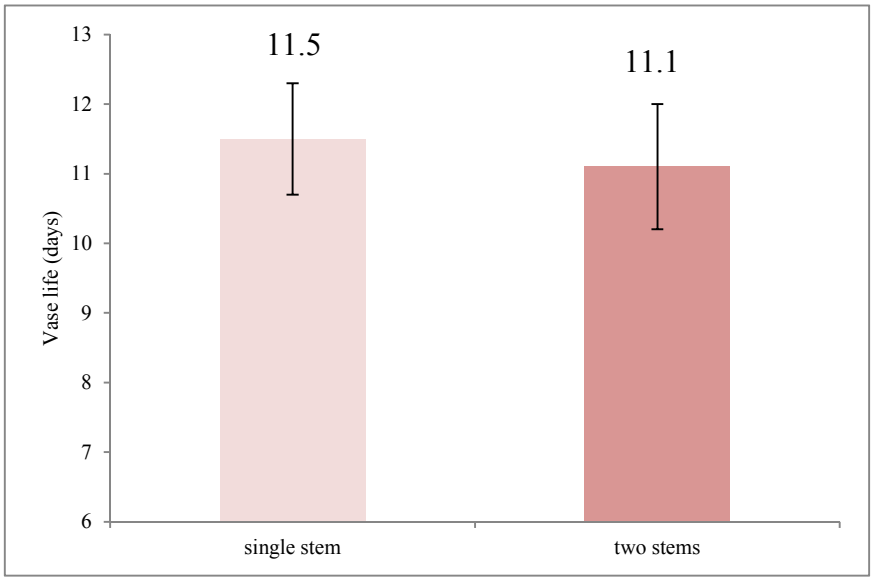


Figure 3.9: Vase life of cut 'Tiber' lilies when placed as single and two stems per vase.

3.3.2 Experiment 3.2: Bacteria populations in vase water and the vase life of the cut 'Akito' rose

3.3.2.1 Changes of bacterial population in vase water of 'Akito' rose.

The 'Akito' rose is a short-lived variety. This experiment was investigated to determine the effect of a number of stems on bacterial population and their vase life. Total bacterial plate count was investigated on TSA. The number of bacteria in the vase water of a single and five stems of 'Akito' rose increased during the first 3 days and then was constant until the end of the vase life (Figure 3.10). The numbers of bacteria in the vase water of a single and five stems were not significantly different throughout the vase life.

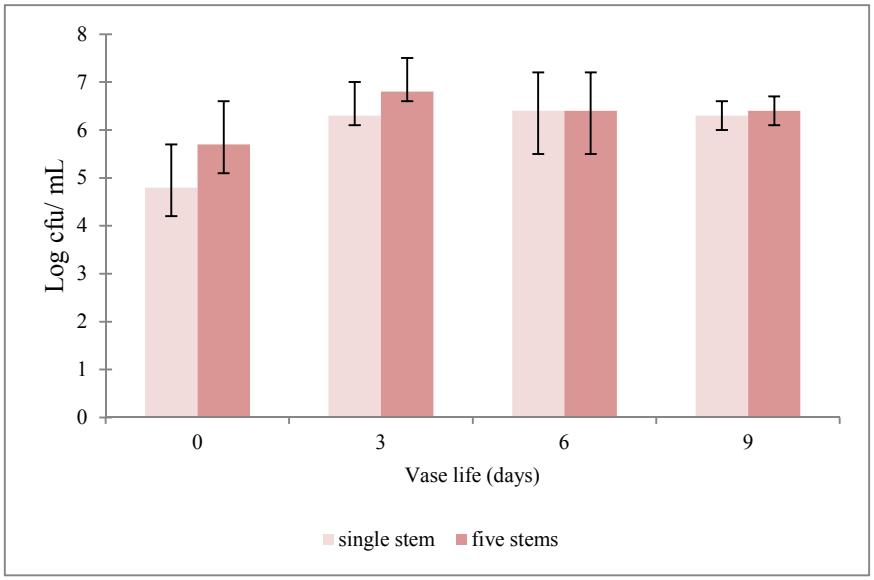


Figure 3.10: Changes of total bacteria plate count of cut 'Akito' roses during the 12 days of vase life. Data are means of three replications, ± SD.

Bacteria that were isolated from this experiment were in the genus of *Neisseria spp*. (A1), *Streptococcus spp.* or *Enterococcus spp.* (A36), and *Enterobacteria spp.* (A55) (Table 3.2). Perhaps surprisingly, no *Pseudomonas spp*. were isolated.

Table 3.2: Unique bacteria found in vase water of 'Akito' roses.

Species	**No.**
Neisseria spp.	A1
Enterococcus spp.	A36
Enterobacteria spp.	A55

Neisseria spp. (A1) were found in this experiment and presented increases in numbers throughout the vase life. There was no difference in numbers of A1 in the vase water of a single and five stems of 'Akito' roses (Figure 3.11).

A36 isolated in this experiment may be in a genus *Enterococcus spp*. The number of A36 in the vase water of a single and five stems of roses increased until day 3 then numbers of A36 were constant. There was no difference in numbers of A36 in the vase water of a single and five stems of 'Akito' roses (Figure 3.12).

For A55 (*Enterobacteria spp*.), the vase water of a single and five stems of 'Akito' roses showed there were no differences in numbers of A55. The numbers of A55 increased from day 0 to day 6 then were constant (Figure 3.13).

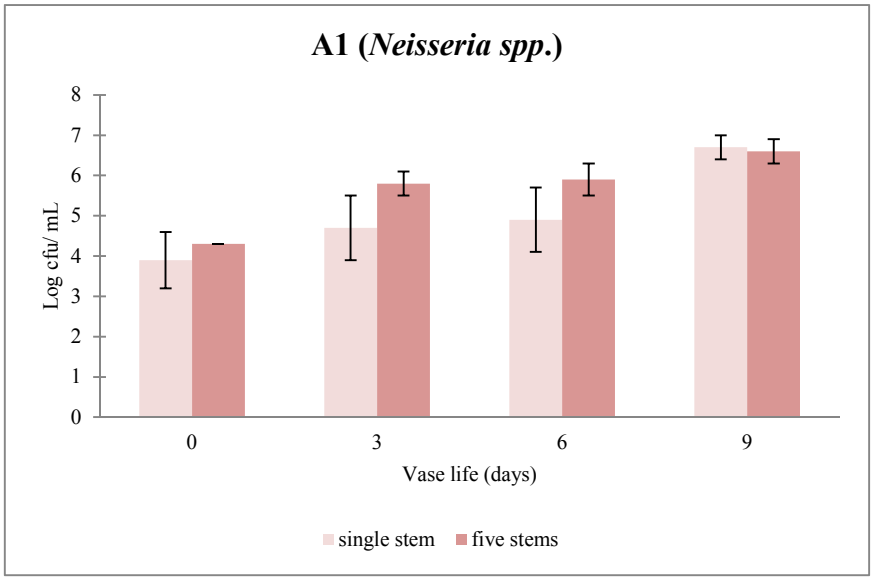


Figure 3.11: Changes of *Neisseria spp*. (A1) of cut 'Akito' roses during the 9 days of vase life. Data are means of three replications, ± SD.

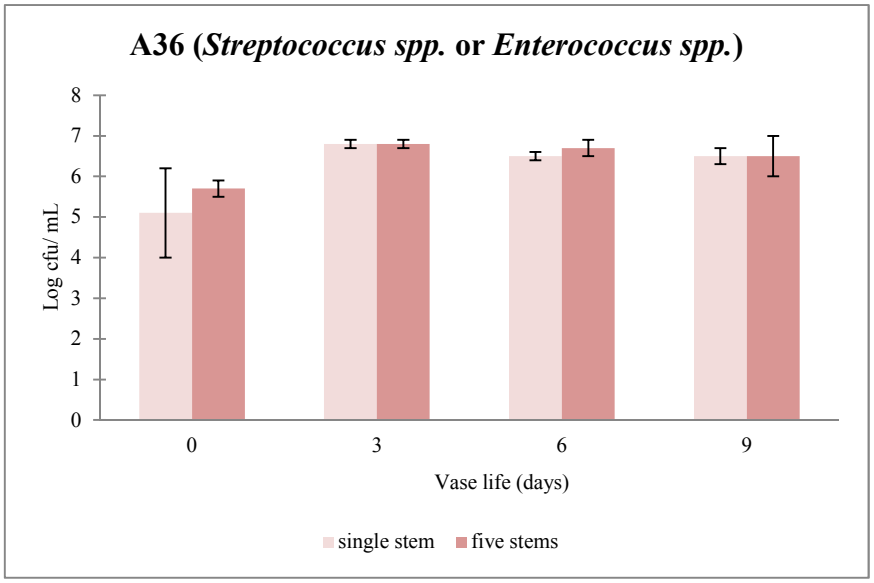


Figure 3.12: Changes of *Streptococcus spp.* or *Enterococcus spp.* (A36) of cut 'Akito' roses during the 9 days of vase life. Data are means of three replications, ± SD.

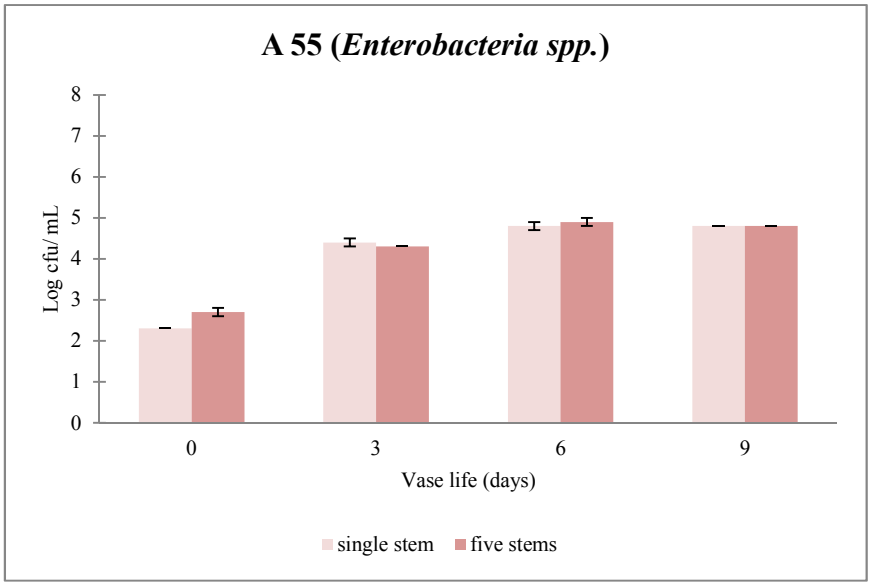


Figure 3.13: Changes of *Enterbacteria spp.* (A55) of cut 'Akito' roses during the 9 days of vase life. Data are means of three replications, ± SD.

3.3.2.2 Changes of water uptake rate, fresh weight and vase life of the 'Akito' rose

Water uptake rate of the 'Akito' roses that were placed as single and five stems started to decrease from day 0 until the end of vase life. The water uptake rate of the single stem declined more slowly than the five stems. The rate of water uptake of the single stem was constant during day 6 to day 9 while the water uptake rate of the five stems decreased throughout the vase life. The water uptake rate of single stems was significantly higher than that of the five stems at day 9 (Figure 3.14).

Fresh weight of 'Akito' roses that were placed with single stems increased their weight initially more than five stems. The fresh weight of the single stem increased until day 3 before declining while the weight of the five stems was constant from day 0 to day 3 then decreased along the vase life. 'Akito' roses which were placed as five stems to a vase lost their weight significantly faster than a single stem (Figure 3.15).

The vase life of 'Akito' roses is shown in figure 3.16. The vase lives of 'Akito' roses that were placed with a single and five stems were 10.8 and 9.1 days, respectively. However, the vase lives of single and five stems were not significantly different.

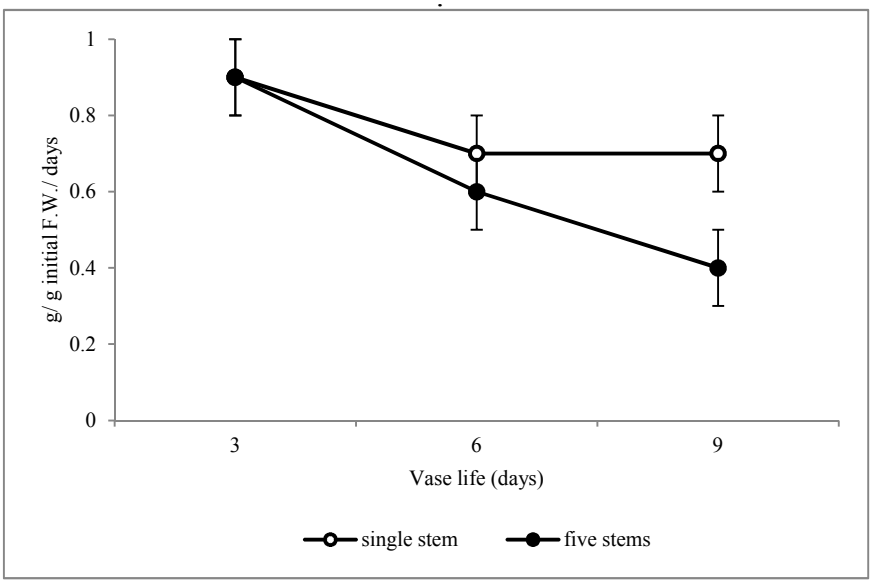


Figure 3.14: Changes of water uptake rate of cut 'Akito' roses during the 9 days of vase life. Data are means of three replications, ± SD.

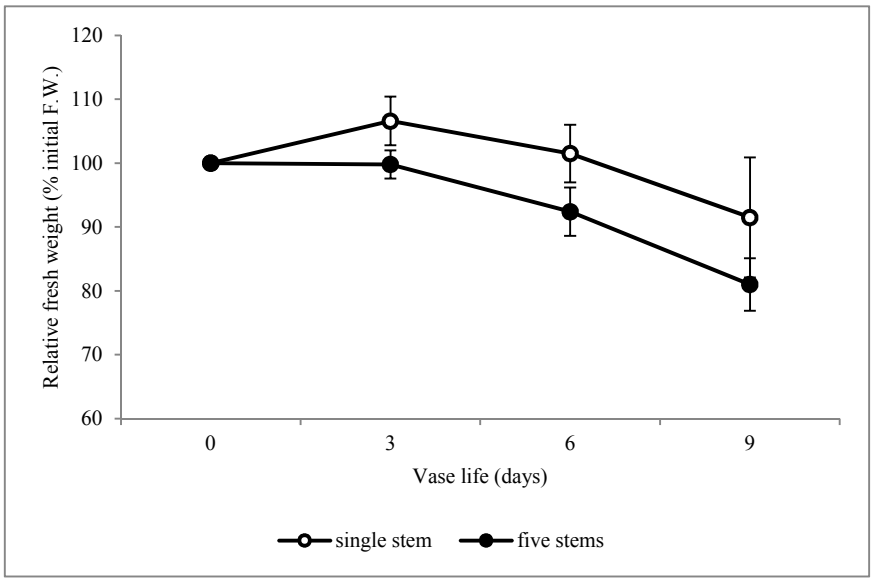


Figure 3.15: Changes of fresh weight of cut 'Akito' roses during the 9 days of vase life. Data are means of three replications, ± SD.

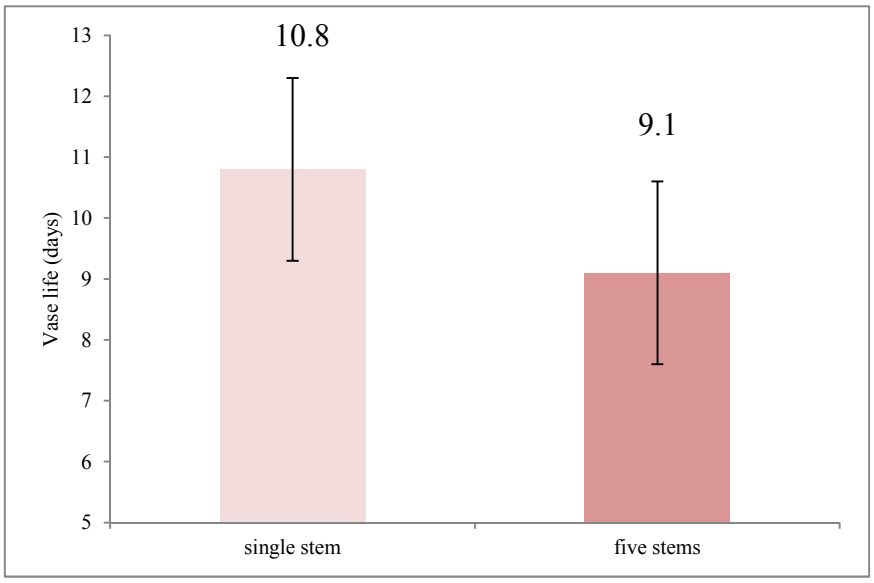


Figure 3.16: Vase life of cut 'Akito' roses when placed as a single and two stems per vase. Data are means of three replications, ± SD.

3.3.3 Experiment 3.3: Bacterial populations in vase water and the vase life of cut 'Valentino' rose.

3.3.3.1 Changes of bacterial population in the vase water of the 'Valentino' rose.

A study of the effect of the number of stems on bacterial population and vase life of a long-lived variety of rose was investigated. The 'Valentino' rose is a long-lived variety which was selected for this study. Data from the total bacterial plate count showed that the bacteria population increased until day 3 and slightly decreased over the vase life (Figure 3.17). The number of bacteria that grew in the vase water of a single and five stems of 'Valentino' roses were not significantly different except on day 3 when the numbers of bacteria in the vase water of five stems were more than those for a single stem.

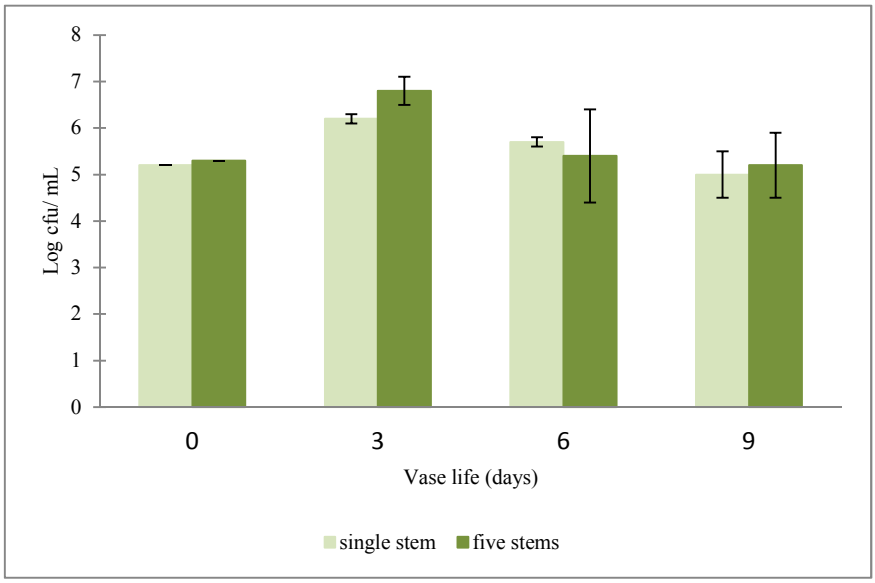


Figure 3.17: Changes of total bacterial plate count of cut 'Valentino' roses during the 9 days of vase life. Data are means of three replications, ± SD.

Investigation into the bacteria in the vase water found that there were three unique bacteria in the genus of *Pseudomonas* spp. (V1), *Bacillus* spp. (V20) and *Staphylococus spp.* (V45) (Table 3.3).

Table 3.3: Unique bacteria found in vase water of 'Valentino' roses.

Species	**No.**
Pseudomonas spp.	V1
Bacillus spp.	V20
Enterococcus spp.	V45

The number of V1 in the vase water of both the single and five stems had the highest number of bacteria at day 6 then slightly declined at day 9. The five stem treatment showed significantly more V1 than the single stem only on day 3 and day 9 (Figure 3.18).

The number of V20 increased throughout the vase life for both single stem and five stem treatments. In the vase water of five stems there were greater numbers of V20 than for the single stem. However, numbers of V20 in the vase water of five stems was significantly more than in the single stem at day 0 and day 6 (Figure 3.19).

Staphylococcusspp. (V45) were found during day 3 to day 9. Numbers of V45 increased until day 6 before they declined at day 9. The single stem treatment had significantly more than in the five stems at day 3 and 9 (Figure 3.20).

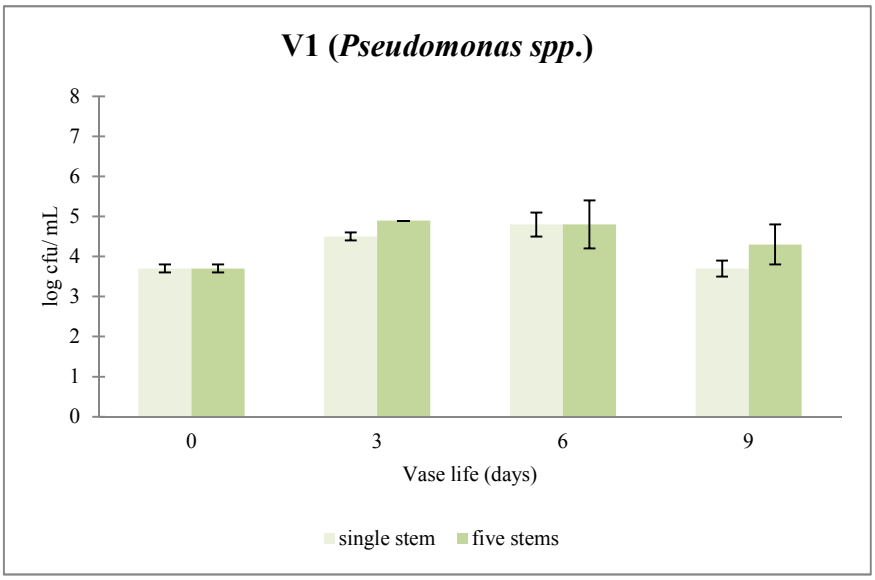


Figure 3.18: Changes of *Pseudomonas spp*. (V1) of cut 'Valentino' roses during the 9 days of vase life. Data are means of three replications, ± SD.

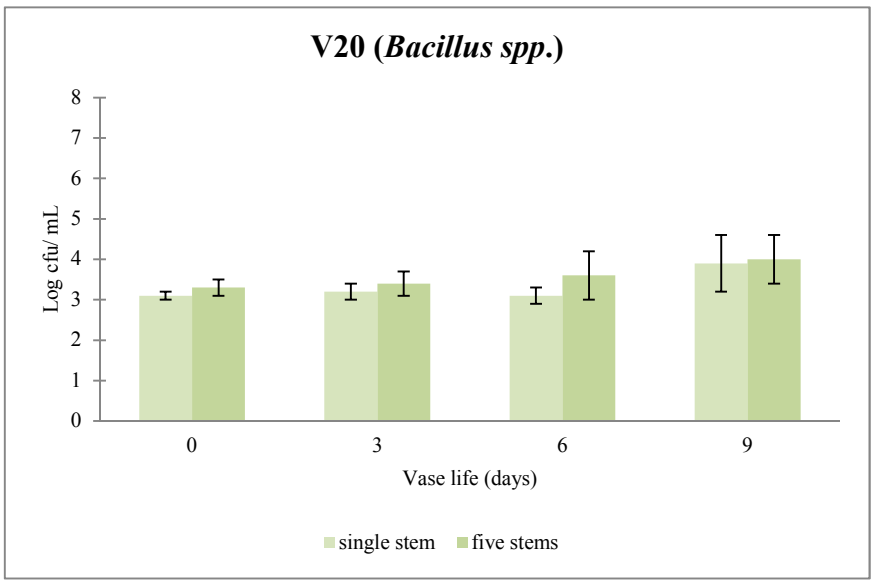


Figure 3.19: Changes of *Bacillus spp*. (V20) of cut 'Valentino' roses during the 9 days of vase life. Data are means of three replications, ± SD.

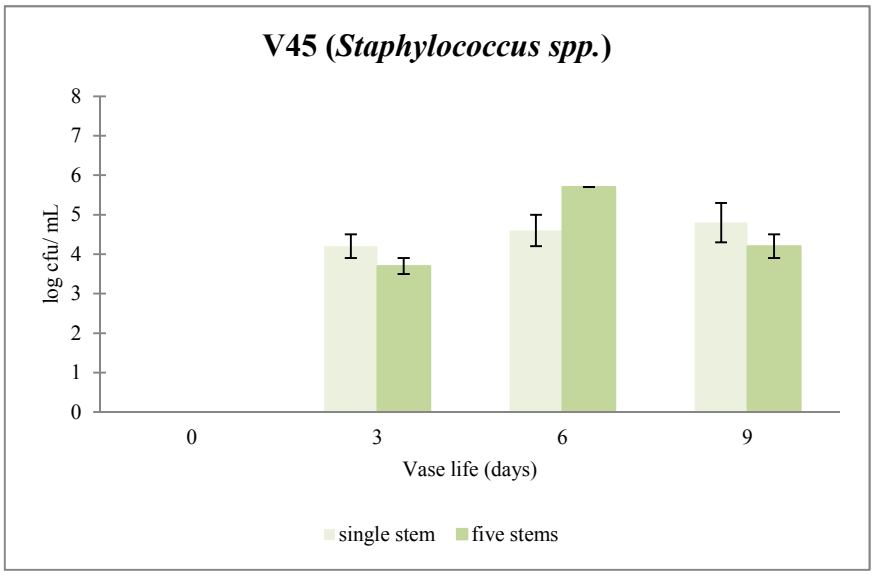


Figure 3.20: Changes of *Staphylococcus spp.* (V45) of cut 'Valentino' roses during the 9 days of vase life. Data are means of three replications, ± SD.

3.3.3.2 Changes of water uptake rate, fresh weight and vase life of the 'Valentino' rose

Changes of water uptake rate of the 'Valentino' roses as a single and five stems both declined throughout the vase life. However, the data of water uptake rate were not significantly different (Figure 3.21).

The fresh weight of the 'Valentino' roses which were placed as a single and five stems increased during the first three days then started to decrease throughout the vase life. However, the data of fresh weight were no different between placing single and five stems in the vase (Figure 3.22).

The vase life of the 'Valentino' roses which were placed with five stems and single stem were not significantly different. The vase lives of a single and five stems were 10.3 and 9.3 days (Figure 3.23).

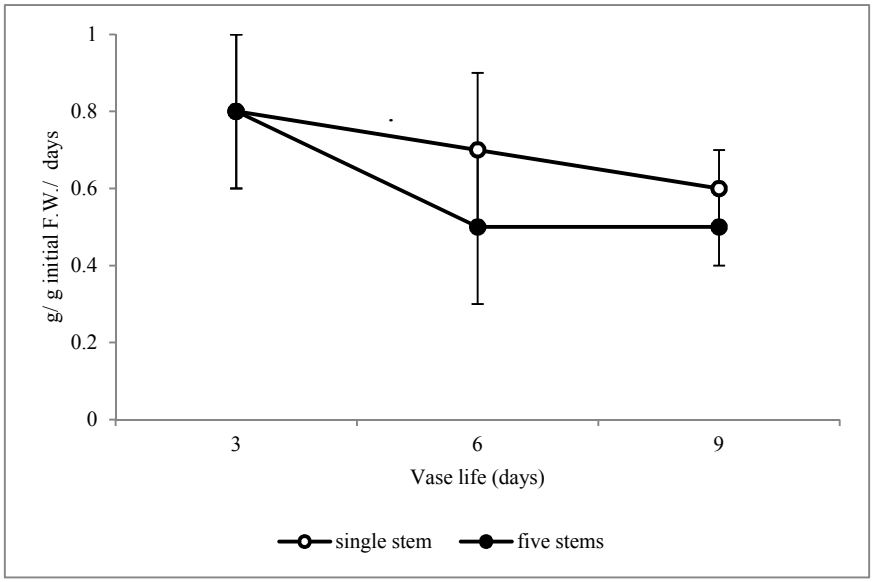


Figure 3.21: Changes of water uptake rate of cut 'Valentino' roses during the 9 days of vase life. Data are means of three replications, ± SD.

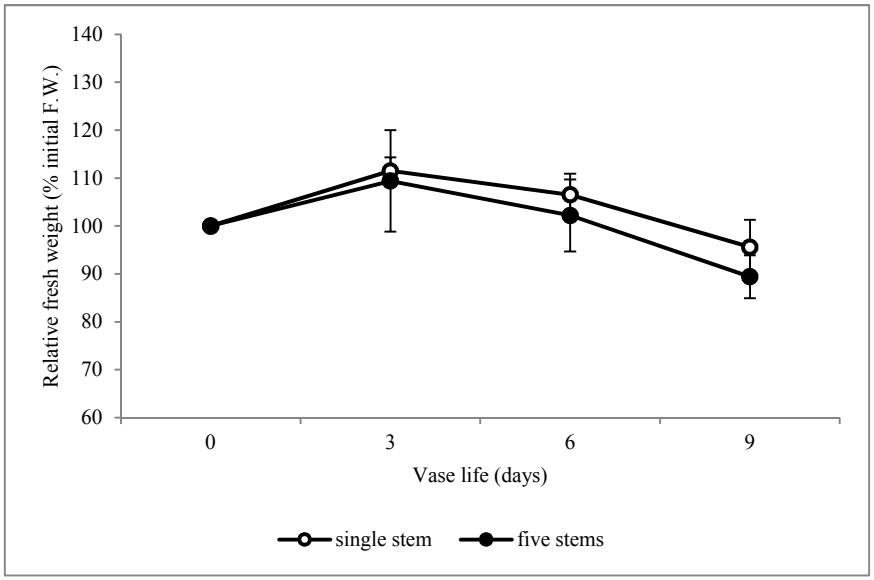


Figure 3.22: Changes of fresh weight of cut 'Valentino' roses during the 9 days of vase life. Data are means of three replications, ± SD.

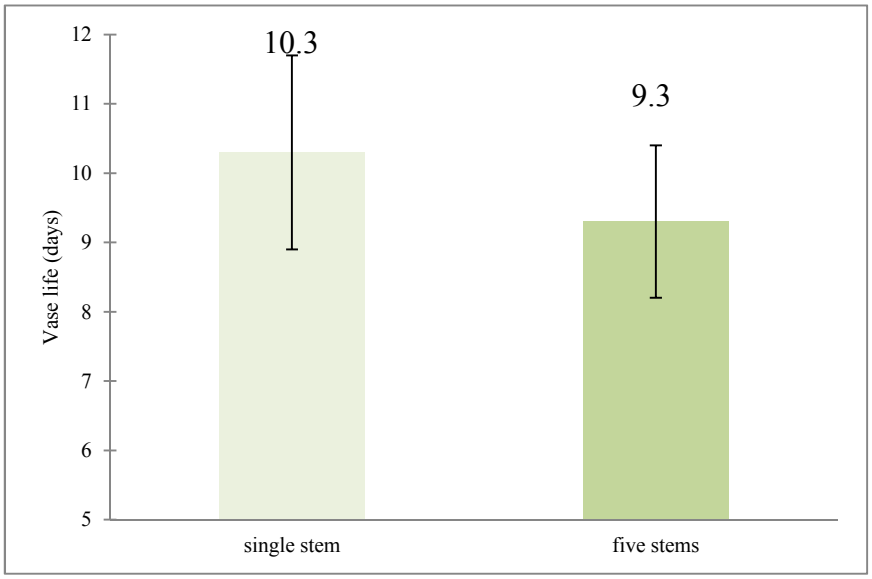


Figure 3.23: Vase life of cut 'Valentino' roses which were placed as a single and two stems per vase. Data are means of three replications, ± SD.

3.3.4 Experiment 3.4: Bacterial populations in vase water and the vase life of a mixed flower bouquets held in distilled water

3.3.4.1 Changes of bacterial population in vase water of mixed flowes bouquets.

The combination of 'Tiber' lily and 'Akito' rose was investigated for bacteria population over 12 days. The vase water used in this first experiment was distilled water. The bacteria population in the vase water was compared between a mixture of single stems of 'Akito' rose with single stems of 'Tiber' lily (1:1 treatment) and a mixture of two stems of 'Tiber' lilies with five stems of 'Akito' roses (2:5 treatment).

The bacterial plate count in the vase water of mixed flowers increased from day 0 to day 12. The vase water of a mixture of 2:5 treatment had a total bacterial plate count greater than the mixture of 1:1 treatment. However, in the vase water of the mixture of 2:5 treatment there was a significantly higher total bacteria plate count than in the mixture of 1:1 treatment after day 9 (Figure 3.24).

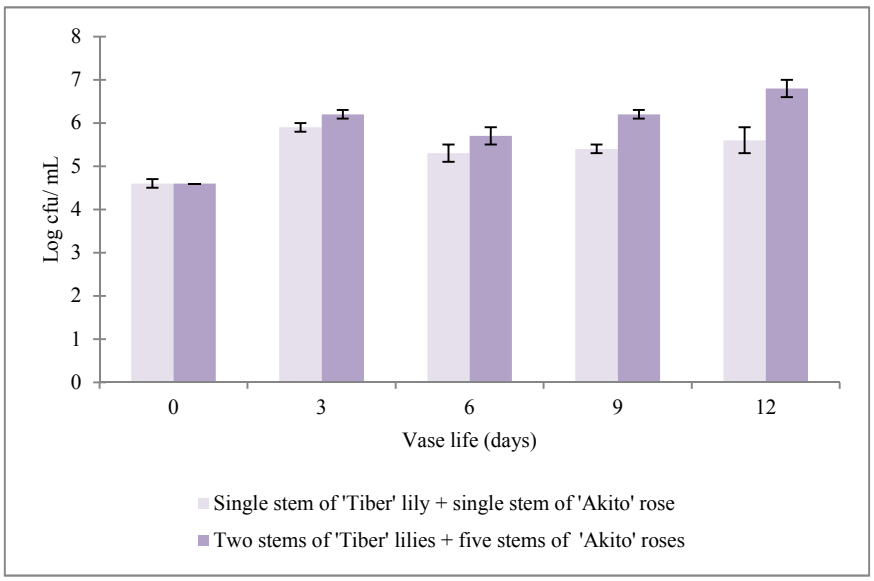


Figure 3.24: Changes of total bacteria plate count of mixed flowers bouquets during the 12 days of vase life. Data are means of three replications, ± SD.

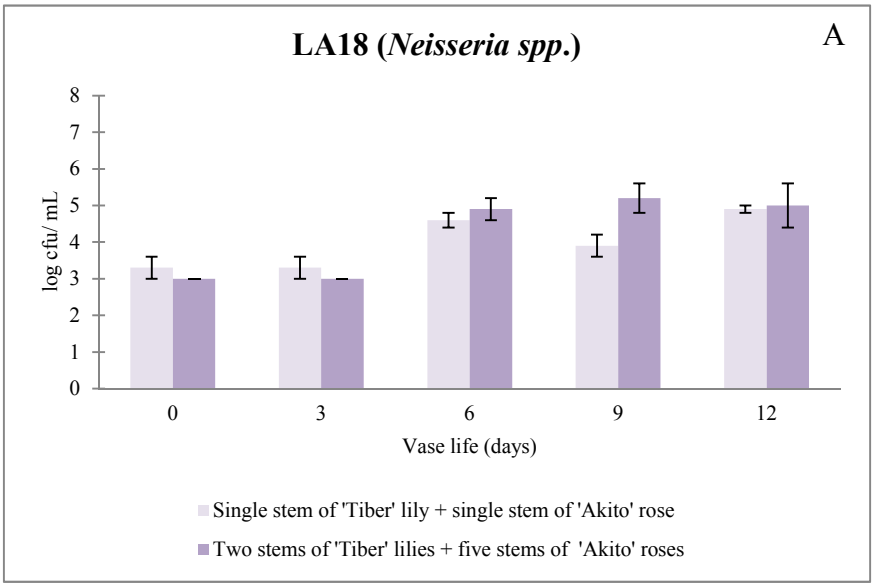
Two unique bacteria were found in this experiment, LA18 and LA45 which belong to the genus *Neisseria* and could be detected during the 12 days of vase life (Table 3.4).

Table 3.4: Unique bacteria found in vase water of mixed flowers bouquets.

Species	**No.**
Neisseria spp.	LA18
	LA45

The number of LA18 in the vase water of mixed flowers increased throughout the vase life. The data for 2: 5 treatment had significantly more LA18 than 1:1 treatment only on day 9 (Figure 3.25 (A)).

The number of LA45 increased from day 0 to day 3 then slightly declined until end of the vase life. There was no difference in the number of LA45 between the two groups of mixed flowers at day 0 and day 3. A mixture of 2:5 treatment had a significantly greater number of LA45 in the vase water than in the vase water of 1:1 treatment after day 6 (Figure 3.25 (B)).



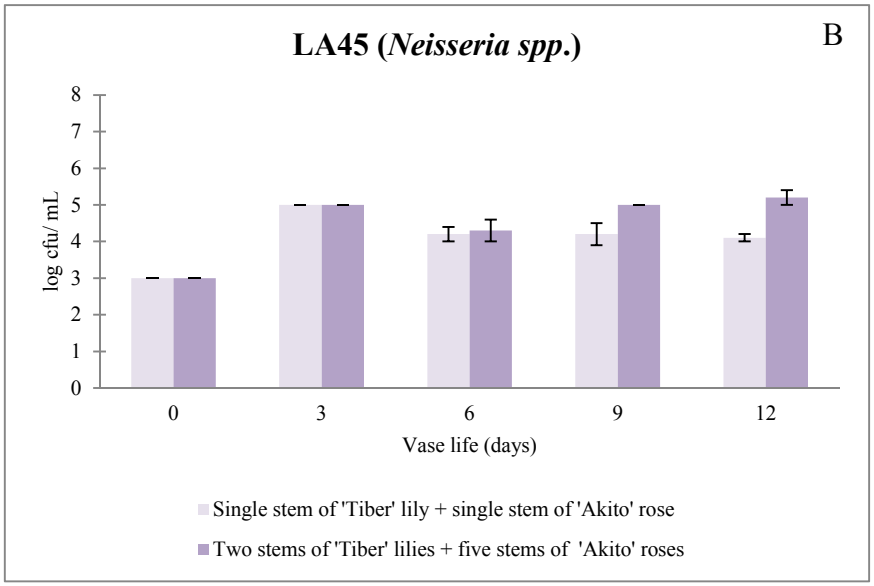


Figure 3.25: Changes of *Neisseria spp*. in the vase water of mixed flowers bouquets held in distilled water during the 12 days of vase life; LA18 (A), LA45 (B). Data are means of three replications, ± SD.

3.3.4.2 Changes of water uptake rate, fresh weight and vase life of mixed flowers bouquets held in distilled water.

Water uptake of mixed flowers slightly decreased throughout the vase life. Water uptake in a mixture of 2:5 declined more than in the mixture of 1:1 treatment after day 6 (Figure 3.26).

Fresh weight of 'Tiber' lilies and 'Akito' roses increased during the first stage of their vase lives then slightly decreased until the end of the vase life. There were differences in fresh weight between placing more and fewer stems in the vase. The fresh weight of 2:5 treatment was lower than in a mixture of 1:1 treatment (Figure 3.27 (A)). While the fresh weight of 'Akito' roses had the same trend in losing their fresh weight, the 'Akito' roses in a mixture of more stems significantly lost more fresh weight than in a mixture of fewer stems throughout the vase life (Figure 3.27 (B)).

The number of stems in the vase had an effect on the vase life of each of the flowers. The vase lives of flowers in a mixture of 1:1 treatment were significantly longer than flowers in a mixture of 2:5 treatment. The vase lives of 'Tiber' lily where placed as a single and two stems in a mixed bunch were 11.5 and 10.3 days respectively. For the 'Akito' rose, the vase lives of the roses in a mixture of single and five stems were 11.5 and 7.6 days respectively (Figure 3.28).

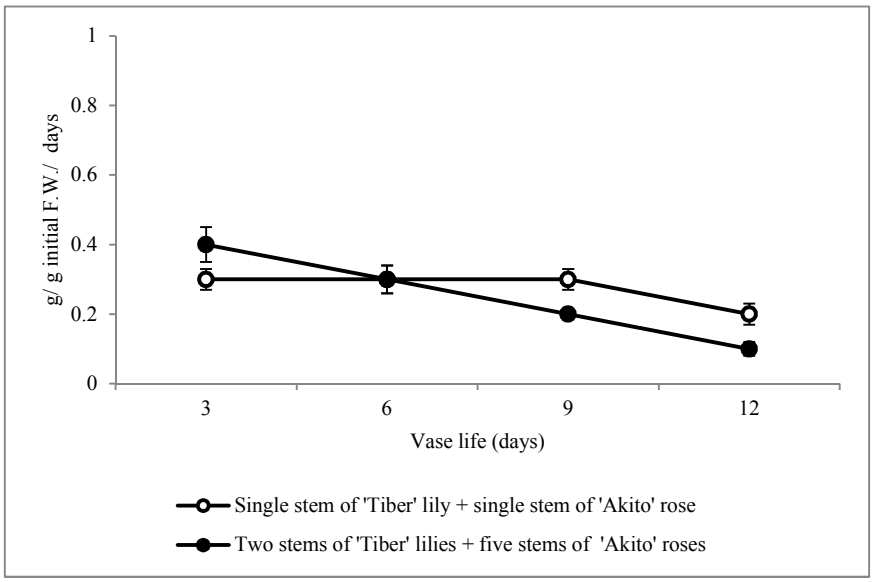
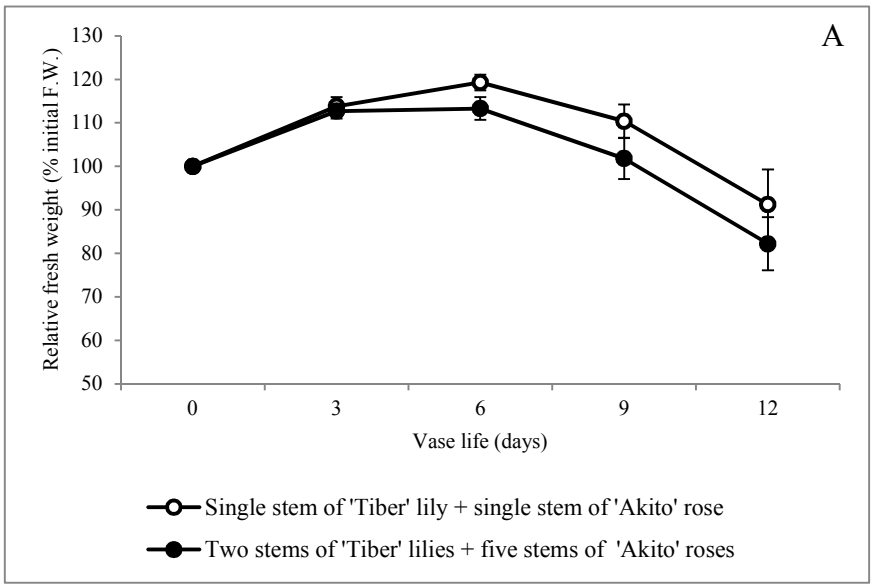


Figure 3.26: Changes of water uptake of cut mixed flowers bouquets during the 12 days of vase life. Data are means of three replications, ± SD.



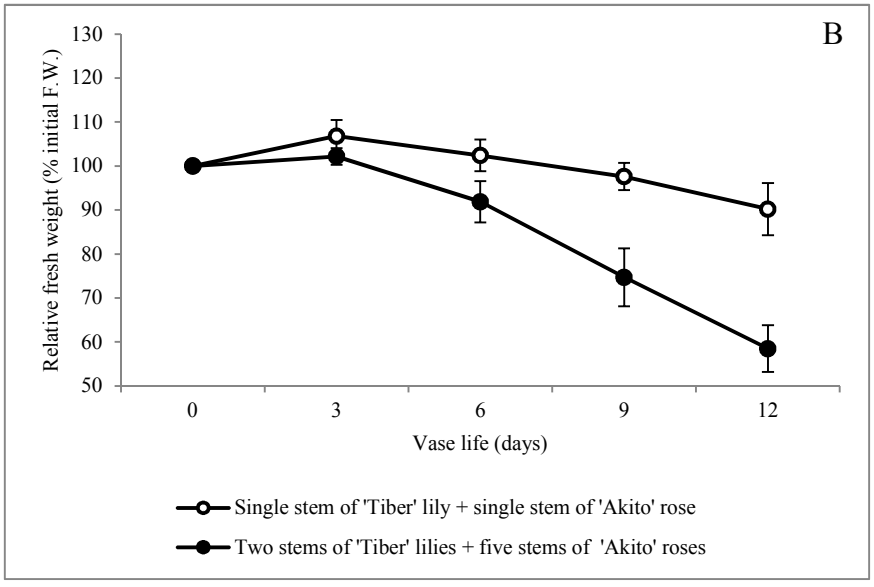


Figure 3.27: Changes of the fresh weight of flowers in mixed flowers bouquets during the 12 days of vase life; 'Tiber' lily (A), 'Akito' rose (B). Data are means of three replications, ± SD.

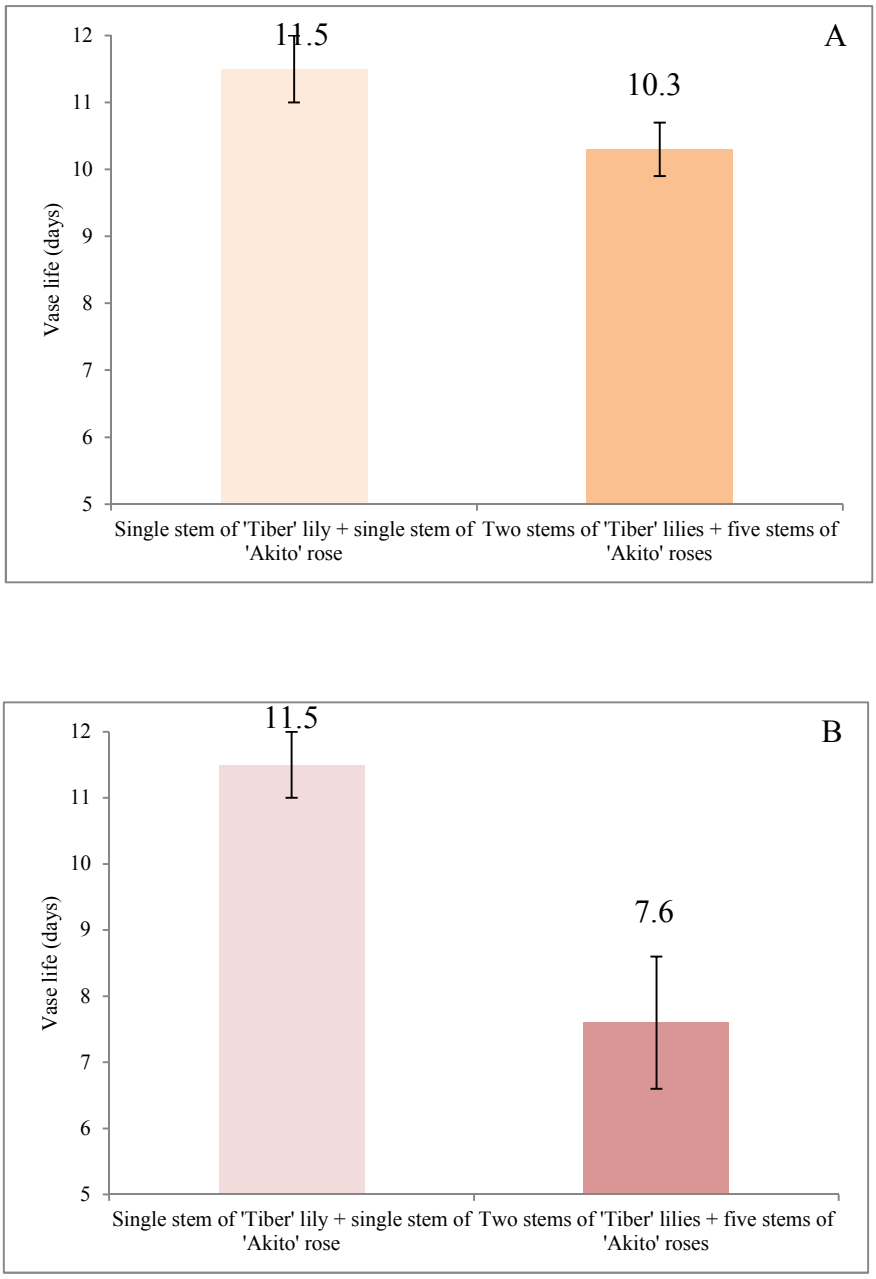


Figure 3.28: Vase life of flowers in mixed flowers bouquets during the 12 days of vase life; 'Tiber' lily (A), 'Akito' rose (B). Data are means of three replications, ± SD.

3.3.5 Experiment 3.5: Bacterial population in vase water and the vase life of mixed flowers bouquets held in flower food

3.3.5.1 Changes of bacterial population in the vase water of mixed flower bouquets.

In this experiment the bacteria population in vase water of mixed 'Tiber' lilies with 'Akito' roses was investigated. The vase water used in this experiment contained a commercial liquid food for roses. A comparison of a mixture of a single stem of 'Tiber' lily with a single stem of 'Akito' rose (1:1 treatment) and two stems of 'Tiber' lilies with five stems of 'Akito' roses (2:5 treatment) was studied over 12 days.

There were greater numbers of bacteria in the vase water at day 0 before the number of bacteria slightly declined throughout the vase life. The total bacterial plate count in this experiment was less than in the experiment described in section 4.4.4. in which a mixture of flowers was held in distilled water. However, data for the bacteria plate count were not significantly different (Figure 3.29).

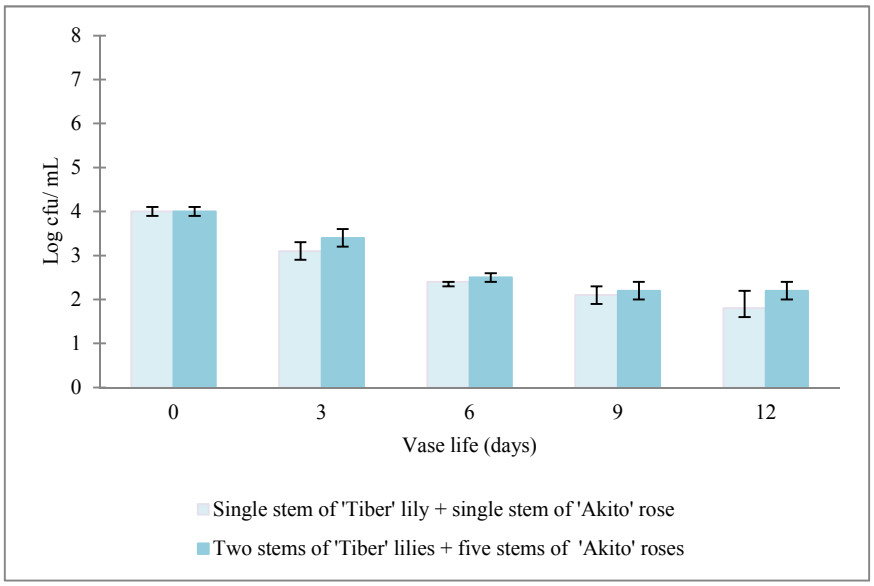


Figure 3.29: Changes of bacteria plate count of mixed flowers bouquets held in flower food during the 12 days of vase life. Data are means of three replications, ± SD.

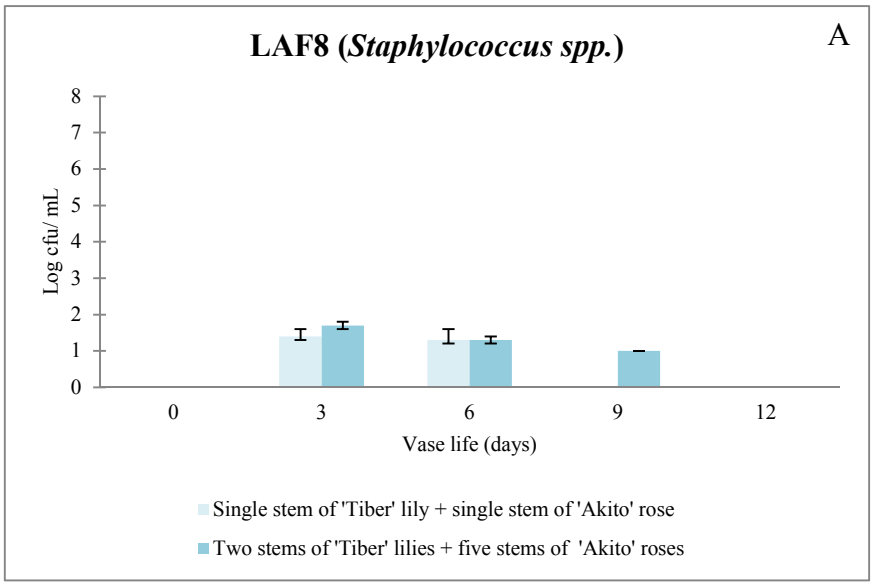
Four unique bacteria were found in this experiment. Two of the bacteria were in genus *Staphylococcus* (LAF8 and LAF10) and the other two belonged to *Neisseria spp*. (LAF13 and LAF18). Most of these were not present over the entire 12 days of vase life (Table 3.5).

Table 3.5: Bacteria found in vase water of 'Valentino' roses.

Species	**No.**
Staphylococcus spp.	LAF8
	LAF10
Neisseria spp.	LAF13
	LAF18

LAF8 was found from day 3 to day 9. It was found in the vase water of the 2:5 treatment only at day 9, but otherwise there was no difference in the number of LAF8 between placing more or fewer stems in vases (Figure 3.30 (A)). Another *Staphylococcus spp.* (LAF10) was evident from day 6 to day 12. The vase water of a mixture of more stems had more LAF10 than fewer stems during day 9 to day 12; on day 12 particularly the vase water in which more stems had been placed had significantly greater numbers of LAF10 than in the vase water in which there were fewer stems (Figure 3.30 (B)).

LAF13 was found in the vase water of mixed flowers only at day 0 and day 3 while LAF18 was found later, from day 6 to day 12. There were no differences in numbers of LAF13 in the vase water when placing more or fewer stems of flowers in the vase. For LAF18, the vase water of 2:5 treatment had significantly more LAF18 than in the vase water of 1:1 treatment on day 12 only (Figure 3.31 (A) and (B)).



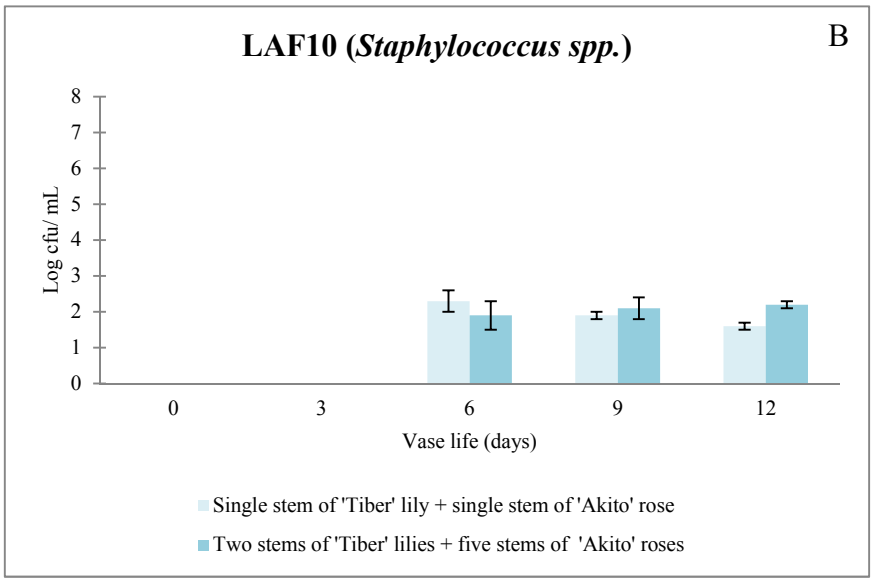
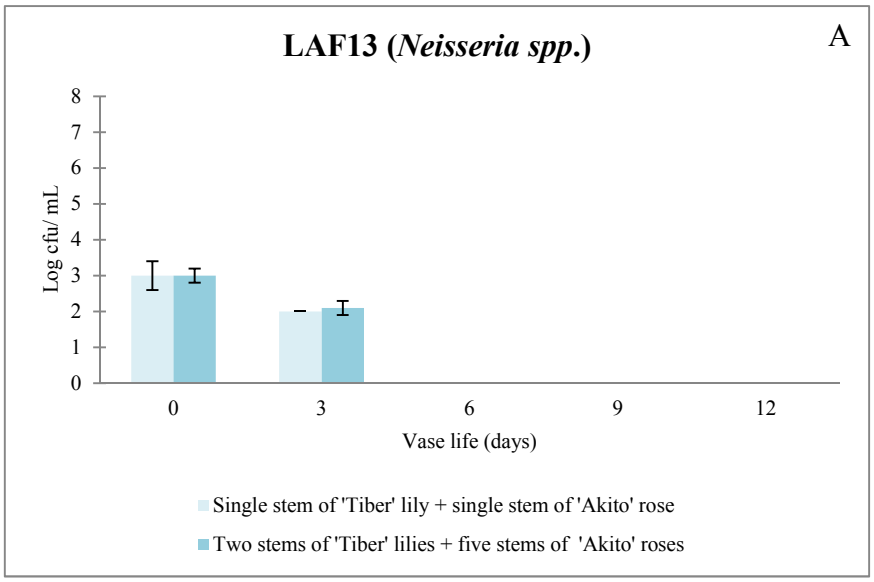


Figure 3.30: Changes of *Staphylococcus spp.* in the vase water of mixed flowers bouquets held in flower food during the 12 days of vase life; LAF8 (A), LAF10 (B). Data are means of three replications, ± SD.



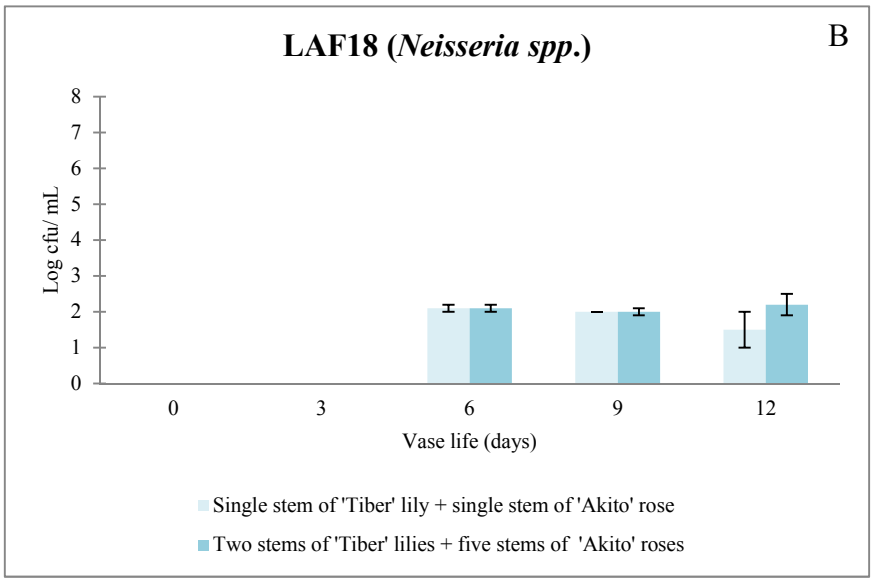


Figure 3.31: Changes of *Neisseria spp*. in the vase water of the mixed flowers bouquets held in flower food during the 12 days of vase life; LAF13 (A), LAF18 (B). Data are means of three replications, ± SD.

3.3.5.2 Changes of water uptake rate, fresh weight and vase life of mixed flower bouquets held in flower food.

Water uptake of flowers in mixed bouquets slightly decreased during day 3 to day 6 then the water uptake was constant. Data of water uptake were not different between placing two stems of 'Tiber' lilies with five stems of 'Akito' roses and placing a single stem of 'Akito' rose with a single stem of 'Tiber' lily (Figure 3.32).

The fresh weight of flowers in mixed bouquets increased during the first 3 days then declined throughout the vase life. However, fresh weight of 'Tiber' lilies and 'Akito' roses in treatment of 1:1 and 2:5 were not significantly different (Figure 3.33 (A) and (B)).

The vase lives of 'Tiber' lilies which were placed singly and two stems in bouquets were not significantly different; their vase lives were 11.5 and 10.9 days respectively. For the 'Akito' rose, the vase lives of the roses in a mixture of a single and five stems were 11.5 and 10.2 days. The vase lives of 'Akito' roses were significantly different with different numbers of stems (figure 3.34 (A) and (B)).

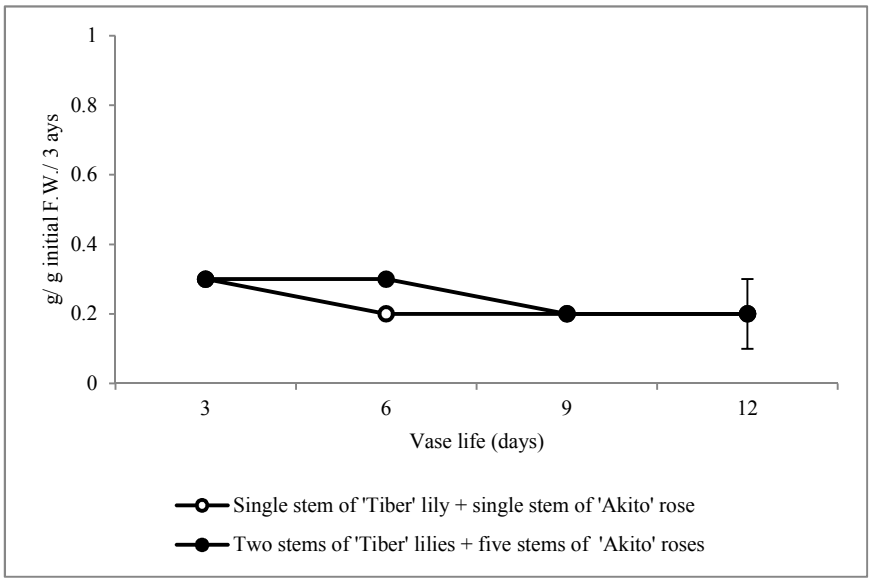
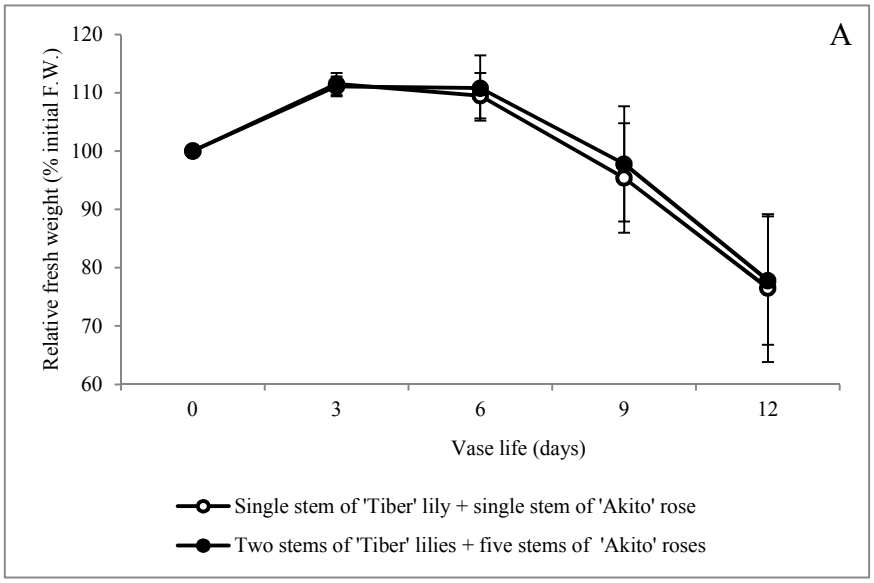


Figure 3.32: Changes of water uptake in the vase water of mixed flower bouquets held in flower food during the 12 days of vase life. Data are means of three replications, ± SD.



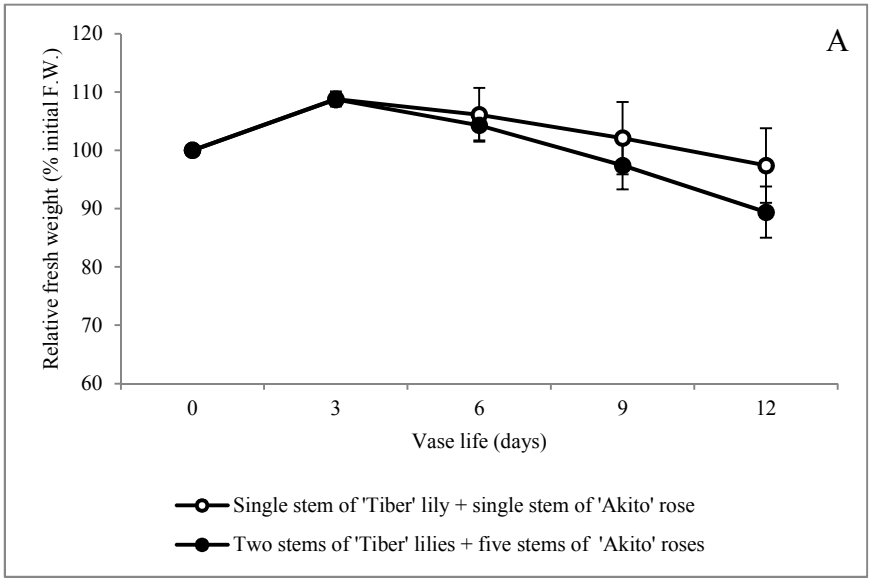


Figure 3.33: Changes of the fresh weight of flowers in mixed flower bouquets held in flower food during 12 days of vase life, 'Tiber' lily (A), 'Akito' rose (B). Data are means of three replications, ± SD.

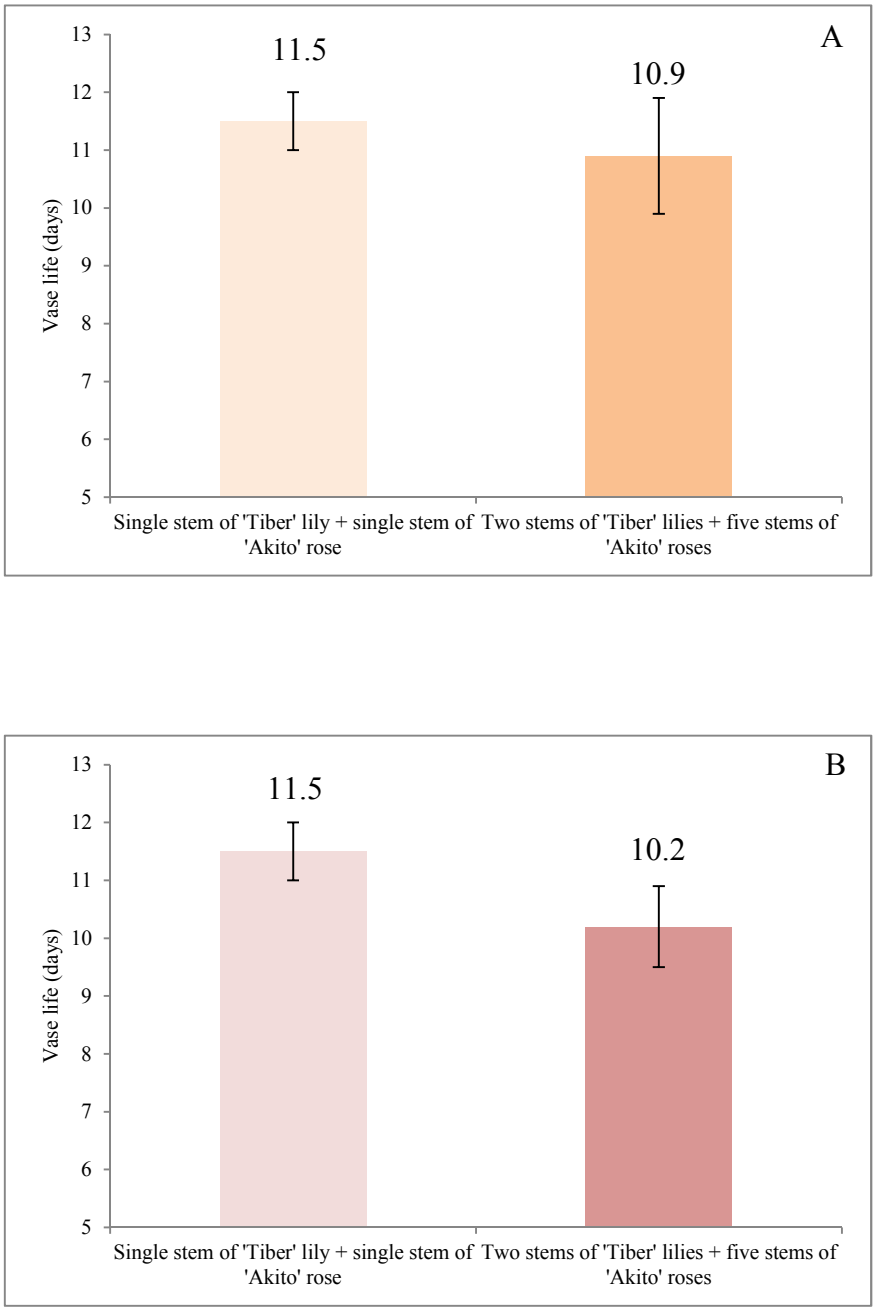


Figure 3.34: Vase life of flowers in mixed flowers bouquets held in flower food during the 12 days of the life, 'Tiber' lily (A), 'Akito' rose (B). Data are means of three replications, ± SD.

3.3.6 Experiment 4.6: Bacteria on the cut surface, outer stems and in xylem vessels of Stems

The bacterial population on the cut surface, outer stems and xylem vessels were evaluated in stems of the 'Tiber' lily and 'Akito' roses. The knowledge of the bacteria in and on the stem may explain that they could act as an inoculum.

Table 3.6: Bacteria on the cut surface of 'Tiber' lily. Data are means of three replications, ± SD.

Position of cut surface (Starting from the cut end)	Bacteria plate count on the cut surface of stems (Log cfu/ cm^2)		
	Day after storage		
	0	6	12
5 cm	ND	5.7 $\pm$ 1.3	5.3 $\pm$ 1.7
10 cm	ND	ND	ND
15 cm	ND	ND	ND
20 cm	ND	ND	ND
25 cm	ND	ND	ND
30 cm	ND	ND	ND

Six positions per stem were observed for the numbers of bacteria on the cut surface of the 'Tiber' lily. Detection of bacteria (day 0) before being placed in distilled water showed that there were no bacteria at all the positions. Bacteria on the cut surface could be detected at days 6 and 12. However, bacteria were found only at 5 cm from the cut end, no bacteria were detected at the other positions above 5 cm. (Table 3.6).

Table 3.7: Bacteria in the cut surface of 'Akito' rose. Data are means of three replications, ± SD.

Position of cut surface (Starting from the cut end)	Bacteria plate count on the cut surface of stems (Log cfu/ $cm^{2)}$		
	Day after storage		
	0	6	12
5 cm	2.4 ± 2.1	9.3 ± 1.2	8.2 ± 1.3
10 cm	ND	4.9 ± 1.9	2.1 ± 1.8
15 cm	ND	ND	ND
20 cm	ND	ND	ND
25 cm	ND	ND	ND
30 cm	ND	ND	ND
35 cm	ND	ND	ND
40 cm	ND	ND	ND
45 cm	ND	ND	ND
50 cm	ND	ND	ND

For the 'Akito' rose, there were 10 positions per stem. Bacteria were detected at 5 cm from the cut end at day 0. After placing the flowers in distilled water for six and 12 days, bacteria could be detected at 5 and 10 cm from the cut end. Bacteria on the cut surface at 5 cm were more numerous than at 10 cm. There were no bacteria at the positions above 10 cm at days 6 and day 12 (Table 3.7).

Table 3.8: Bacteria on the outer stem of 'Tiber' lily. Data are means of three replications, ± SD.

Position of stem (Starting from the cut end)	Bacteria plate count on the cut surface of stems (Log cfu/ cm^2)		
	Day after storage		
	0	6	12
0-5 cm (under the water)	0.1 ± 0.0	0.3 ± 0.0	0.3 ± 0.0
5-10 cm (under the water)	ND	0.3 ± 0.0	0.3 ± 0.0
10-15 cm	ND	0.2 ± 0.1	0.2 ± 0.0
15-20 cm	ND	0.2 ± 0.1	0.1 ± 0.0
20-25 cm	ND	0.1 ± 0.1	0.2 ± 0.1
25-30 cm	ND	ND	ND

Bacteria on the outer stems were observed on six pieces of the 'Tiber' lily's stem. The length of each piece was 5 cm. Bacteria were detected at 0-5 cm at day 0 before being held in distilled water while on the other pieces above 5 cm bacteria could not be detected. The level of distilled water in the vase was 10 cm in height. The pieces of stems that were under the distilled water, were 0-5 cm and 5-10 cm. Bacteria on the outer stem were detected starting at 0-25 cm from the cut end at day 6 and day 12. The parts of the stems that were under the distilled water had a greater number of bacteria than the pieces above the water (Table 3.8).

Table 3.9: Bacteria on the outer stem of 'Akito' rose. Data are means of three replications, ± SD.

Position of stem (Starting from the cut end)	Bacteria plate count on the outer stems (Log CFU/ cm^2)		
	Day after storage		
	0	6	12
0-5 cm (under the water)	0.3 ± 0.1	0.3 ± 0.0	0.4 ± 0.0
5-10 cm (under the water)	ND	0.3 ± 0.0	0.4 ± 0.0
10-15 cm	ND	0.3 ± 0.0	0.4 ± 0.0
15-20 cm	ND	0.4 ± 0.0	0.3 ± 0.0
20-25 cm	ND	0.3 ± 0.2	0.3 ± 0.1
25-30 cm	ND	0.1 ± 0.1	0.2 ± 0.1
30-35 cm	ND	ND	ND
35-40 cm	ND	ND	ND
40-45 cm	ND	ND	ND
45-50 cm	ND	ND	ND

For the 'Akito' rose, 10 pieces of stem were evaluated for the number of bacteria. The length of each piece was 5 cm. Before being held in distilled water (day 0), bacteria were found only at 0-5 cm from the cut end. The height of distilled water in the vase was 10 cm. The pieces of stems under the distilled water, were 0-5 cm and 5-10 cm. At day 6 and day 12 after placing the flowers in distilled water, bacteria on the outer stem were detected at 0-30 cm from the cut end. For the length of stem from 0-25 cm, numbers of bacteria did not differ significantly between different positions. However, the pieces at 25-30 cm had progressively fewer numbers of bacteria, while for those pieces that were higher than 30 cm no bacteria could be detected at day 6 and day 12 (Table 3.9)

Table 3.10: Bacteria in xylem of the stem of 'Tiber' lily. Data are means of three replications, ± SD.

Position of stem (Starting from the cut end)	Bacteria plate count in xylem (Log CFU/ cm^3)		
	Day after storage		
	0	6	12
0-5 cm	1.3 ± 0.3	2.1 ± 0.0	2.3 ± 0.1
5-10 cm	1.1 ± 0.4	1.8 ± 0.2	1.8 ± 0.1
10-15 cm	ND	1.3 ± 0.2	1.3 ± 0.3
15-20 cm	ND	0.7 ± 0.4	0.7 ± 0.4
20-25 cm	ND	ND	0.7 ± 0.4
25-30 cm	ND	ND	ND

Bacteria in xylem of the 'Tiber' lily were evaluated from six pieces of stem. The length of each piece was 5 cm. At day 0, bacteria were detected at 0-10 cm from the cut end while on the pieces above 10 cm bacteria could not be detected. Bacteria were evaluated again at day 6 and day 12 after the flowers were held in distilled water. Bacteria were detected from 0-20 cm at day 6 and from 0-25 cm at day 12. The base of the stem had the highest number of bacteria with progressively fewer at the higher positions (Table 3.10).

Table 3.11: Bacteria in xylem of the stem of 'Akito' rose. Data are means of three replications, ± SD.

Position of stem (Starting from the cut end)	Bacteria plate count in xylem (Log CFU/ cm^3)		
	Day after storage		
	0	6	12
0-5 cm	1.2 ± 0.5	2.9 ± 0.1	3.2 ± 0.3
5-10 cm	ND	2.3 ± 0.2	2.6 ± 0.3
10-15 cm	ND	2.2 ± 0.2	1.4 ± 0.4
15-20 cm	ND	1.1 ± 0.6	1.1 ± 0.5
20-25 cm	ND	ND	0.8 ± 0.3
25-30 cm	ND	ND	0.2 ± 0.0
30-35 cm	ND	ND	ND
35-40 cm	ND	ND	ND
40-45 cm	ND	ND	ND
45-50 cm	ND	ND	ND

Bacteria in xylem of 'Akito' rose were evaluated from 10 pieces of stem. The length of each piece was 5 cm. At day 0, bacteria were detected at 0-5 cm from the cut end while on the pieces above 5 cm bacteria could not be detected. After holding the flowers in distilled water, bacteria were found on the stem outer surfaces from 0-20 cm at day 6 and at 0-30 cm at day 12. The base of the stem had the highest number of bacteria with progressively fewer at the higher positions. The number of bacteria in the xylem at 0-10 cm increased with storage (Table 3.11).

3.3.7 Summary of bacterial species and the effect of difference in numbers of stems on the vase life of cut flowers

Table 3.12 shows a summary of bacteria that were found in the vase water of 'Akito' roses, 'Valentino' roses, and a mixture of 'Tiber' lilies and 'Akito' roses held in distilled water and flower food. This study found seven groups of bacteria overall in the vase water that was observed for single flower species and in mixtures of flowers.

Table 3.12: Summary of bacteria found in the vase water of 'Akito' roses, 'Valentino' roses, and a mixture of 'Tiber' lilies and 'Akito' roses held in distilled water and liquid flower food.

Species	**No**.	**Plant**				
		'Tiber' lily	'Akito' rose	'Valentino' rose	Mixture of 'Tiber' lilies with 'Akito' roses (Distilled water)	Mixture of 'Tiber' lilies with 'Akito' roses (Flower food)
Staphylococcus spp.	L7	√				
	L8	√				
	LAF8					√
	LAF10					√
Streptococcus spp. or *Enterococcus spp.*	L9	√				
	A36		√			
	V45			√		
Bacillus spp.	V20			√		
Enterobacteria spp.	L4	√				
	A55		√			
Pseudomonas spp.	L15	√				
	L29	√				
	V1			√		
Brucella spp.	L10	√				
Neisseria spp.	A1		√			
	LA18				√	
	LA45				√	
	LAF13					√
	LAF18					√

The experiment with ‘Tiber’ lily found two gram positive bacteria in the group of *Staphylococcus.* The experiment with ‘Tiber’ lilies and ‘Akito’ roses held in liquid flower food also found two bacteria in the *Staphylococcus group*. However, bacteria manifested in both experiments were not the same species.

For gram positive bacteria in the group of *Streptococcus* or *Enterococcus*, they emerge in the single variety experiments of ‘Tiber’ lily, and ‘Akito’ and ‘Valentino’ roses. However, they were not the same species. Gram positive bacteria in the group of *Bacillus* were found in only one species in the experiment of ‘Valentino’ rose.

Gram negative bacteria in the group of *Enterobacteria* were found in single variety experiments of ‘Tiber’ lily and ‘Akito’ rose. However, they were not the same species. For gram negative bacteria in the group of *Pseudomonas,* two species were found in the single variety experiments of ‘Tiber’ lily and one species in ‘Valentino’ rose. However, they were not the same species.

This experiment found gram negative bacteria in the group of *Neisseria*. These were present in the single variety experiments of ‘Akito’ rose, and were also manifested in both mixture experiments that were held in distilled and liquid flower food. However, they were also not the same species.

In the experiment of mixed flowers, a mixture of ‘Tiber’ lilies with ‘Akito’ roses held in distilled water, a bacterium in the group of *Neisseria* was found. Mixed flowers held in flower food found a bacterium in the group of *Staphylococcus*. Moreover, mixed flowers held in flower food also found group of *Neisseria.*

Table 3.13: Total bacteria plate count, species of bacteria, vase life, fresh weight and water uptake of 'Tiber' lilies at the end of vase life.

Treatment		Total bacteria count (Log cfu/ mL)	Group of bacteria	Vase life	Fresh weight (% initial F.W.)	Water uptake (g/ g initial F.W./ day)
Single variety	Single stem	5.4	*Staphylococcus spp.* (L7, L8), *Streptococcus spp.* or *Enterococcus spp.* (L9), *Enterobacteria spp.* (L4), *Pseudomonas spp.* (L15, L29), *Brucella spp* (L10).	11.5	88.6	0.3
	Two stems	5.4	*Staphylococcus spp.* (L7, L8), *Streptococcus spp.* or *Enterococcus spp.* (L9), *Enterobacteria spp.* (L4), *Pseudomonas spp.* (L15, L29), *Brucella spp* (L10).	11.1	93.7	0.2
Mixed flower	Lily: Rose ; 1:1 (distilled water)	5.6	*Neisseria spp.* (LA18, LA45)	11.5	91.2	0.2
	Lily: Rose; 2:5 (distilled water)	6.8	*Neisseria spp.* (LA18, LA45)	10.3	82.2	0.1
	Lily: Rose; 1:1 (flower food)	1.8	*Staphylococcus spp.* (LAF8, LAF10), *Neisseria spp.*(LAF13, LAF18)	11.5	76.5	0.2
	Lily: Rose; 2:5 (flower food)	2.2	*Staphylococcus spp.* (LAF8, LAF10), *Neisseria spp.*(LAF13, LAF18)	10.9	77.8	0.2

Table 3.13 shows a summary of the main results obtained from experiments with the 'Tiber' lily, both alone and in mixtures with the 'Akito' rose. Total bacterial plated count at the end of vase life showed that bacterial populations in the vase water of the mixed flowers held in distilled water were higher than for a single variety and mixed flowers held in flower food. Increasing stems of 'Tiber' lily alone did not increase the number of bacteria present. In mixtures, where an overall increase in bacterial number was observed, this was not reflected in the individual data for all bacterial species, and differences often only became significant at the end of the experiments. A mixture of 1:1 treatment had a total bacterial plate count less than in the 2:5 treatment.

Surprisingly, a more varied bacterial flora was evident in the single variety experiments, compared to mixed variety experiments. Bacteria in the group of *Neisseria spp.* were found in mixed flowers held in both distilled water and flower food.

The vase lives of the 'Tiber' lily when placed as single and two stems, in the single variety experiment were not different. The vase lives of lilies in a 1:1 mixture treatment held in distilled water and flower food were the same as those of single stems in the single variety experiments. While the vase lives of the 2:5 treatment were shorter than with the 1:1 treatment.

Surprisingly, the relative fresh weight of mixed flowers held in liquid flower food was lower than in mixed flowers held in distilled water and in the single variety experiments. The 2:5 treatment of mixed flowers held in distilled water was lower than the 1:1 treatment. Water uptake of two stems of the single variety experiment and the 2:5 treatment of the mixed flowers held in distilled water were lower than the single stem and 1:1 treatment. In the mixed flowers' experiment held in flower food, water uptakes of the 1:1 and 2:5 treatments were not different.

Table 3.14: Total bacteria plate count, species of bacteria, vase life, fresh weight and water uptake of 'Akito' roses at the end of vase life.

Treatment		Total bacteria count (Log cfu/ mL)	Group of bacteria	Vase life	Fresh weight (% initial F.W.)	Water uptake (g/ g initial F.W./ day)
Single variety	Single stem	6.3	*Streptococcus spp.* or *Enterococcus spp.* (A36), *Enterobacteria spp.*(A55), *Neisseria spp*. (A1)	10.8	91.5	0.7
	Five stems	6.4	*Streptococcus spp.* or *Enterococcus spp.* (A36), *Enterobacteria spp.*(A55), *Neisseria spp*. (A1)	9.1	81.0	0.4
Mixed flower	Lily: Rose ; 1:1 (distilled water)	5.6	*Neisseria spp.* (LA18, LA45)	11.5	90.2	0.2
	Lily: Rose; 2:5 (distilled water)	6.8	*Neisseria spp.* (LA18, LA45)	7.6	58.5	0.1
	Lily: Rose; 1:1 (flower food)	1.8	*Staphylococcus spp.* (LAF8, LAF10), *Neisseria spp.*(LAF13, LAF18)	11.5	97.4	0.2
	Lily: Rose; 2:5 (flower food)	2.2	*Staphylococcus spp.* (LAF8, LAF10), *Neisseria spp.*(LAF13, LAF18)	10.2	89.4	0.2

The total bacterial plate count at the end of vase life showed that bacterial populations in the vase water of single and five stems in the experiment with the single variety were not different. The total bacterial plate count of the 2:5 treatment was more than the 1:1 treatment of mixed flowers held in distilled water and flower food. However, differences were only significant towards the end of the experiments. As expected, the total bacterial plate count of the mixed flowers experiment held in flower food was less than for those held in distilled water and the single variety experiments.

As in the data for 'Tiber' lily, the experiment with the single variety found more groups of bacteria than in the mixed flowers experiments. Bacteria in the groups of *Staphylococcus* and *Neisseria* were found in the single variety experiments and were also found in the mixed flower experiments held in both distilled water and liquid flower food.

The vase life of 'Akito' roses using five stems was shorter than for single stems. The experiment with mixed flowers found the same trend. The vase life of the 2:5 treatment was shorter than for the 1:1 treatment.

For fresh weight, 'Akito' roses placed as five stems in a single variety experiment had fresh weight lower than the single stem. The results in mixed flowers experiments showed the same trend as the single variety experiments. Fresh weight of the 2:5 treatment was lower than that of the 1:1 treatment.

Placing more stems into vases had an influence on water uptake. Water uptake of five stems of the single variety experiment and 2:5 treatment of mixed flowers held in distilled water were lower than for single stems and the 1:1 treatment. In the mixed flowers experiment held in liquid flower food, water uptake of 1:1 and 2:5 treatments were not different.

3.4 Discussion

3.4.1 The type of bacteria found in the vase water of a single variety and mixtures of flowers

This study investigated bacteria in the vase water of single variety and mixed flowers.

Previous researches found both gram positive and gram negative bacteria similar to those found in this study, especially *Pseudomonas*, which has frequently been isolated from the vase water of cut flowers (Put, 1990), and was also isolated on a number of occasions in this study. In fact, most bacteria found in this experiment were similar to those seen in previous research. There were 19 species of bacteria found in the vase water of 'Tiber' lily, 'Akito' rose, 'Valentino' rose, and mixed bouquets of 'Tiber' lilies and 'Akito' roses. They were in the following groups: *Staphylococcus, Streptococcus* or *Enterococcus, Bacillus*, *Enterobacteria, Pseudomonas, Brucella*, and *Neisseria*.

Bacteria in group of *Bacillus, Enterobacteria* and *Pseudomonas* were similar to previous research that reported these groups to be generally found in cut flowers e.g. chrysanthemum, gerbera and rose (Put, 1990; Kates *et al*., 1991; van Doorn and de Witte, 1991; van Doorn *et al*., 1991). Moreover, van Doorn *et al*. (1991) reported *Pseudomonas* and *Enterobacter* were always found at the cut surface and in the xylem vessels. *Pseudomonas spp*. were more frequently emerged in vase water than *Enterobacter spp*., due to the fact that *Pseudomonas spp*. do not require organic growth factors. *Enterobacteriaceae* often need more growth requirements. Their growth was increased after the leakage of carbohydrates (glucose, fructose, sucrose) and proteins from the damaged plant tissue (Put, 1990).

In the experiments on single variety flowers, *Staphylococcus spp., Streptococcus spp.* or *Enterococcus spp., Bacillus spp., Enterobacteria spp., Pseudomonas spp.* and *Brucella spp.*, were isolated from the vase water of 'Tiber' lilies. For 'Akito' roses, *Streptococcus spp.* or *Enterococcus spp., Enterobacteria spp.* and *Neisseria spp*. were found in vase water, while *Streptococcus spp. or*

Enterococcus spp., Bacillus spp. and *Pseudomonas spp.* were isolated from 'Valentino' roses.

In addition, these experiments found a high proportion of other bacteria in the group of *Streptococcus* or *Enterococcus, Brucella* and *Neisseria*, which have previously been isolated less often in vase water. In general, a high proportion of the bacteria found in individual experiments was unique to those particular flower varieties. In particular, in the experiments with mixed flowers, bacterial species found were not the same as those found in the single variety experiments. Perhaps surprisingly, there was a more varied micro-flora in the single variety experiments than was found in the mixed variety experiments, which may reflect the conditions in the vase water exerted by the mixed stems (perhaps including antimicrobial compounds). Therefore, differences between species of bacteria may be due to differences between plants. Van Doorn *et al*, (1991) suggest that the composition of the bacteria at the cut end and inside the xylem of cut 'Sonia' roses is similar to that found in vase water. Put (1990) found that bacteria which were isolated from cut flower stems are normal inhabitants of agricultural soil, and that these are the species which develop in the vase water.

However, Put (1990) has suggested that the development of microorganisms in vase water was influenced also by the unique ecological conditions in the vase rather than by the microorganisms present on the stems.

One of the most striking points about the current work was the very high number of bacteria present from the beginning of the experiments. This indicates that the stems themselves contributed strongly to the flora present in the vases. However, in most cases the bacteria which developed from this appeared to be specific to the stems present in the vase, which suggests that ecological conditions have a strong influence also.

3.4.2 Effect of number of stems on bacterial populations and vase life

This experiment studied the effect of numbers of stems on bacterial population and vase life. The mixed flower bouquets studied in this research were a mixture of two stems of 'Tiber' lilies with five stems of 'Akito' roses. Difference in numbers of stems was compared in both single variety and mixed flowers.

For ‘Tiber’ lily, it was found that there were no differences in terms of total bacteria plate count and vase life for single or two stems. However, water uptake of the two stems treatment was lower than for a single stem. This is an interesting result because the initial count was surprisingly high, indicating that stems provide a high number of bacteria into the vase water, and therefore the population present should be highly dependent on stem number. This may indicate that the numbers of bacteria on stems are highly variable.

For cut roses, the differences in numbers of stems were studied using the ‘Akito’ rose (short-lived cultivar) and ‘Valentino’ rose (long-lived cultivar). The influence of numbers of stems on bacterial populations and the vase life of both cultivars had the same trend as for the ‘Tiber’ lily. The vase water of the five stem treatment overall appeared to show the same had more total bacteria plate count as for the single stem treatment, although individual bacterial species showed some significant differences at various stages of the experiments. However, the vase life of the five stems treatment was shorter than that of the single stem treatment, which may indicate that there are other factors which influence vase life, apart from bacterial populations. Again, there were high numbers of bacteria from the start of the experiment, and the influence of the stem number is unclear. It may be that only a proportion of the bacteria present in these experiments were culturable under the conditions imposed, in which case the effect of adding more stems would have a less direct effect on the numbers seen overall. Water uptake and fresh weight of five stems treatment was also lower than single stems. The results from single variety experiments with ‘Tiber’ lily, ‘Akito’ rose and ‘Valentino’ rose, indicated that increasing more stems in a vase increases bacteria populations, especially when total counts are considered. However, increasing stem numbers certainly reduced vase life, especially for the roses. Due to the high number of bacteria generally present, the accumulation of bacteria in the vase solution and on cut stems must still be considered to be a major factor limiting the longevity of cut flowers and foliage (Ratnayake *et al*., 2012).

Studies on the effect of numbers of stems in mixed flowers experiments were investigated using distilled water and liquid flower food. The results showed that a mixture of two stems of ‘Tiber’ lilies with five stems of ‘Akito’ roses (2:5 treatment)

had more total bacterial plate counts than a mixture of single stems of 'Tiber' lily and 'Akito' rose (1:1 treatment). There were more bacteria populations in the 2:5 treatment, which could be partly responsible for the reduced vase life of both the 'Tiber' lily and 'Akito' rose. This treatment had the highest number of bacteria recorded at the end of the experiment, compared to all other experiments conducted, but as has been noted earlier, the diversity of the population was more restricted than for some other experiments, notably those with single varieties. Adding more stems of 'Akito' roses had a greater effect on vase life, fresh weight and water uptake than in 'Tiber' lilies. This indicated that the 'Akito' roses had a greater response to high bacteria populations than the 'Tiber' lilies.

However, the vase life of 'Akito' roses was extended by using liquid flower food. The liquid flower food for roses was used in this experiment. Total bacterial populations in the experiment with liquid flower food were lower than in distilled water. Generally, liquid solutions for cut flowers usually consist of germicides or bactericides, a carbohydrate, surfactants, acidifiers (Teixeira da Silva, 2003). So it is to be expected that the bacterial populations will be diminished in this treatment. However, bacterial populations were not eliminated in this treatment. In general, the trend for the total microbial count was that it diminished with time, while some species disappeared and others appeared later in the study (as shown in the individual species data).

Therefore, added stems in the vase were related to size of bacterial population. In cut rose flowers, vase life is usually limited due to water stress symptoms such as wilting and bent neck. The development of these symptoms may be due to xylem blockage (Mayak *et al*., 1974). This in turn may be related to an increase in the number of bacteria at the cut surface and in the xylem vessels (van Doorn *et al*., 1989; van Doorn, 1991, 1997; Bleeksma and van Doorn, 2003).

This experiment investigated the number of bacteria on the cut surface, outer surface and in the xylem of the 'Tiber' lily and 'Akito' rose. There were more bacterial populations at the 5 cm position from the basal cut end in both lilies and roses. These results were similar to those of van Doorn (1991) who found bacteria accumulation located only very close to the cut end. Van Doorn and de Witte (1991) reported that blockage in cut rose stems was located in the basal 5 cm of the stem.

There was correlation between a decrease in hydraulic conductance in the xylem and high numbers of bacteria.

3.5 Conclusion

This experiment investigated bacteria in vase water of single variety and mixed flowers. There were 19 species of bacteria in total found in the vase water of 'Tiber' lily, 'Akito' rose, 'Valentino' rose, and mixed flowers of 'Tiber' lilies and 'Akito' roses. They were in the groups of *Staphylococcus, Streptococcus* or *Enterococcus, Bacillus, Enterobacteria, Pseudomonas, Brucella*, and *Neisseria*. These species have previously been found by other researchers.

Bacteria found in all experiments seemed to be largely specific to different flower types. Also, species of bacteria found in the vase water of mixed flowers were less than in for a single variety. Difference in species of bacteria may be due to differences between plants, and the conditions they exert in the vase water. In general bacterial numbers were high at the beginning of all the experiments indicating a strong inoculum effect from stems, although there was not a straightforward relationship between stem numbers and inoculum size, in all cases.

Experiments of a single variety were studied in 'Tiber' lily, 'Akito' rose and 'Valentino' rose. The results indicated that the addition of stems did not result in higher numbers of bacteria, but that vase life was reduced, especially in roses.

The effect of the number of stems in mixed flowers was investigated in distilled water and liquid flower food. The results showed that mixtures of two stems of 'Tiber' lilies with five stems of 'Akito' roses (2:5 treatment) had a higher total bacterial plate count than a mixture of single stems of 'Tiber' lily and 'Akito' rose (1:1 treatment). The result of the addition of stems was to reduce vase life of both the 'Tiber' lily and 'Akito' rose. However, the effect of more stems of 'Akito' roses had a greater effect on vase life, fresh weight and water uptake than for 'Tiber' lilies. This indicated that 'Akito' roses had more response to high bacteria populations than lilies.

This experiment investigated the number of bacteria on the cut surface, outer surface and in the xylem of the ‘Tiber’ lily and ‘Akito’ rose. The results showed that there were more bacteria at 5 cm above the cut end in both lilies and rose.

CHAPTER FOUR

Influence of chemical exudates from 'Tiber' lily and 'Akito' rose stems on the vase lives of flowers in mixed bouquets

4.1 Introduction

Previous research reported that the quality of water has an influence on quality and vase life of cut flowers. Brecheisen *et al.* (1995), reported water quality as having an effect on prolonged flower quality. Plant tissue, such as the cell sap of xylem, contains various cations, anions, and solutes of organic and inorganic matter. These chemicals can leak from plant tissue to vase water (van Meeteren *et al.,* (2000); Xie *et al.,* (2008)). Chemical exudates passing from flower stems into water may have an effect on other flowers. In 2004, van Doorn *et al.,* reported that narcissus flowers could delay the senescence of iris flowers by placing them in the same vase. They also reported that Narciclasine in daffodil mucilage could inhibit the synthesis of proteins involved in senescence. This suggests that chemical exudates from flower stems may have a beneficial effect on vase life in some instances.

Besides the effects of bacterial population on vase life, there has generally been little research into the effects of chemical exudates in mixed bouquets, and none on the flower types used in the current studies. This author's project considers if chemical exudates from the stems of various flowers held together in the same vase may have an effect on the vase lives of each flower. The mixture of two stems of 'Tiber' lilies and five stems of 'Akito' roses were observed for their chemical exudate interaction without an influence of bacterial population (at least initially). The results of this experiment may clarify the role of chemical exudates on the vase life of cut flowers.

4.2 Materials and methods

4.2.1 Plant material

'Akito' roses and 'Tiber' lilies at the commercial stage were obtained from Flamingo Holdings Company Ltd., (Gt North Road, Sandy, Bedfordshire SG19 2AJ).

Then all the flowers were transported to the Microbiology Laboratory at Cranfield University, UK. After that, they were selected for the same uniformity and as being without defects. Stem lengths of all the samples were re-cut to 50 cm under water using a sterile razor blade (van Doorn, 1997). Leaves on the lower one-third of the stems were stripped. Within each stem of the lilies, the number of buds per stem was kept as constant as possible for determining the stem vase life. To attempt to eliminate bacterial populations (at least from external surfaces), all stems of flowers were cleaned with 50 ppm DICA (Sodium dichloroisocyanuric acid) before being held in the water.

4.2.2 Experiment design

- Experiment 1: A single stem of 'Tiber' lily was stood in a 2-Litre vase containing 200 ml of sterile distilled water, sterile old water from five stems of 'Akito' rose and non-sterile old water from five stems of 'Akito' rose.
- Experiment 2: A single stem of 'Akito' rose was stood in a 2-Litre vase containing 200 ml of sterile distilled water, sterile old water from two stems of 'Tiber' lily and non-sterile old water from two stems of 'Tiber' lily.

The experiment was a completely randomised design (CRD) with five replications. A CRD was adopted in the vase life room. Each vase of flowers was placed in the vase life room at 20°C and given a 12 hour on-off cycle under a lamp throughout the vase life.

4.2.3 Preparation of vase water and samples for microorganism estimation

4.2.3.1 Preparation of vase water

Vase water used to investigate the effect of chemical exudates from the stems of 'Tiber' lily and 'Akito' rose was collected from previous storage of two stems of 'Tiber' lilies and five stems of 'Akito' roses. The vase water was kept at -20°C until required.

To destroy bacteria without any effect on chemical exudates, distilled water (control) and the sterile old water treatment were filtered through a cellulose nitrate membrane sterilization unit (0.2 µm pore) (Thermo Scientific Nalgene).

4.2.3.2 Preparation of samples for microorganism estimation

Vase water from five replications per treatment was sampled after stirring and 3 ml from each sample aseptically pipetted into a sterile bottle. Samples from the vases were also taken aseptically before placing the flowers in the vases and at the end of the vase life. A protection against contamination was achieved by sealing a plastic film to the top of each vase.

One mL of vase water were diluted into 10 to 1,000- fold dilutions , and 100 µL of vase water were spread on sterile Tryptone Soya Agar (Oxoid), The inoculated plates were incubated at 25°C for two days before counting.

4.2.4 Physiological measurements

4.2.4.1 Vase life

The cut flower longevity was recorded as days of vase life from the time the flowers were placed into the vases (day 0). The end of the vase life was indicated when a score of flower quality reached stage 5 (refer to flower standard criteria in chapter 2).

4.2.4.2 Fresh weight and water uptake measurement

The stem fresh weight (f.w.) and the vase weight (vase + water) were measured at the same time every day from day 0. These data were used to determine the relative fresh weight (RFW) as a percentage of the initial fresh weight (% initial f.w.) and water usage in millilitres per day.

4.2.4.3 Time to open of the primary bud and final stage of bud opening

Time to open of the primary bud of 'Tiber' lily was calculated as days up until full opening. For final stage of bud opening of 'Akito' rose, was also calculated (refer to flower standard criteria in chapter 2)

4.2.5 Statistical analysis

Significance tests were made by analysis of variance (ANOVA) using SPSS version 16 was applied to water uptake, relative fresh weight, total bacterial plate count, time to open of the primary bud, final stage of bud opening and vase life. Mean comparisons were made using least significance difference (LSD).

4.3 Results

4.3.1 Investigation of chemical exudates from 'Akito' rose stems on the vase life of the 'Tiber' lily

Water uptake of the 'Tiber' lily held in different vase waters was slightly decreased until day 5 then slightly increased and was then constant until the end of the vase life. The water uptakes of all treatment were significantly different after day 7 (Figure 4.1).

The percentage of fresh weight of the 'Tiber' lilies increased until day 5 then they started to lose their weight. The fresh weight of the 'Tiber' lilies held in sterile distilled water was significantly more than those held in sterile old water and non-sterile old water at day 1 and day 3. However, the fresh weights of 'Tiber' lilies of all treatments were not significantly different after day 3 (Figure 4.2).

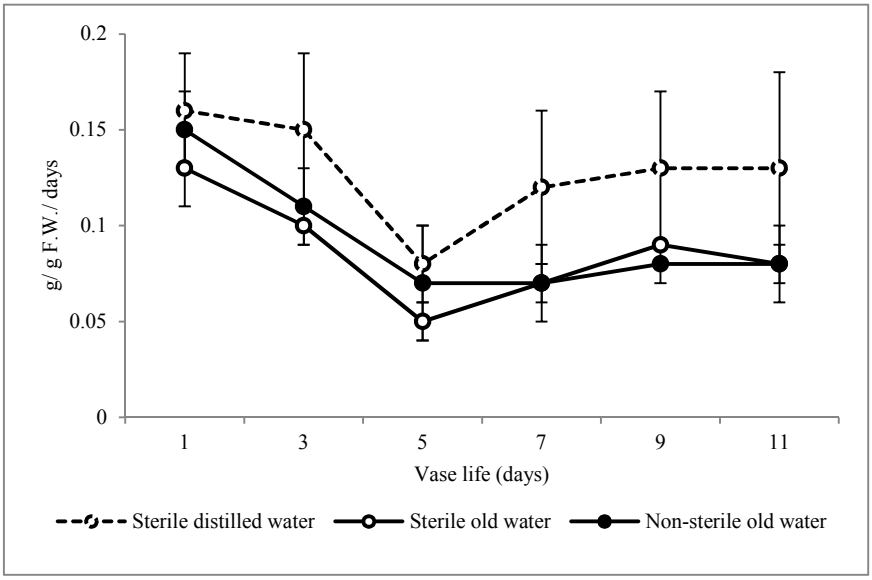


Figure 4.1: Changes of water uptake of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water during the 12 days of vase life. Data are means of three replications, ± SD.

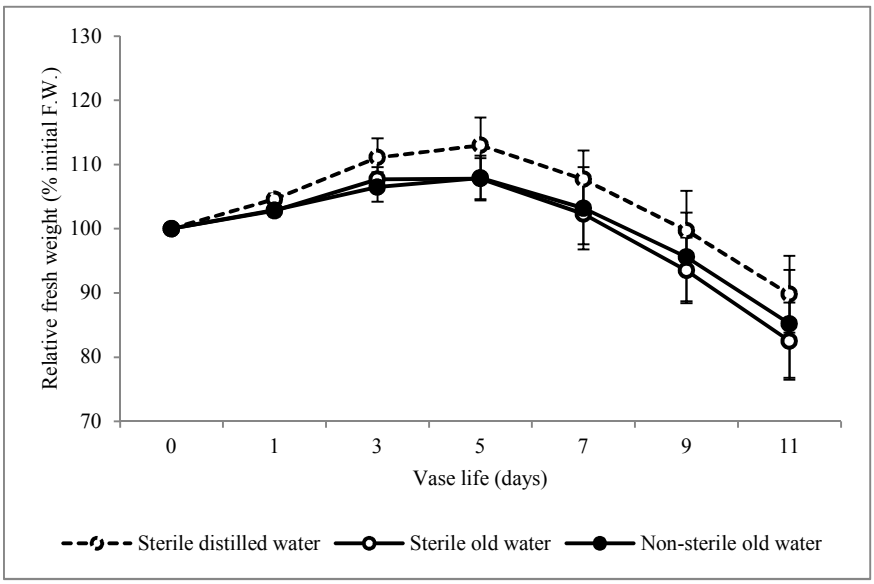


Figure 4.2: Changes of fresh weight of the 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase water during the 12 days of vase life. Data are means of three replications, ± SD.

Table 4.1: Time to opening of the first bud and vase life of 'Tiber' lily

Treatment	Time to opening of the first bud (days)	Vase life (days)
Sterile distilled water	5.8^{NS}	11.4^{NS}
Sterile old water	6.0^{NS}	11.3^{NS}
Non-sterile old water	6.4^{NS}	10.4^{NS}

When comparing time to opening of the primary bud of the 'Tiber' lily, time to opening of the primary bud of the 'Tiber' lily flowers held in sterile distilled water, sterile old water and non-sterile old water was 5.8, 6.0 and 6.4 days, respectively. However, data were not significantly different (Table 4.1)

Investigation into the vase life of all treatments showed that the vase life of lilies held in sterile distilled water, sterile vase water and non-sterile vase water was 11.4, 11.3 and 10.4 days, respectively (Table 4.1). Vase lives of all treatments were not significantly different.

The changes in overall appearance of the 'Tiber' lilies are shown in Figure 4.3. Lily flowers bud opening did not appear different between treatments.

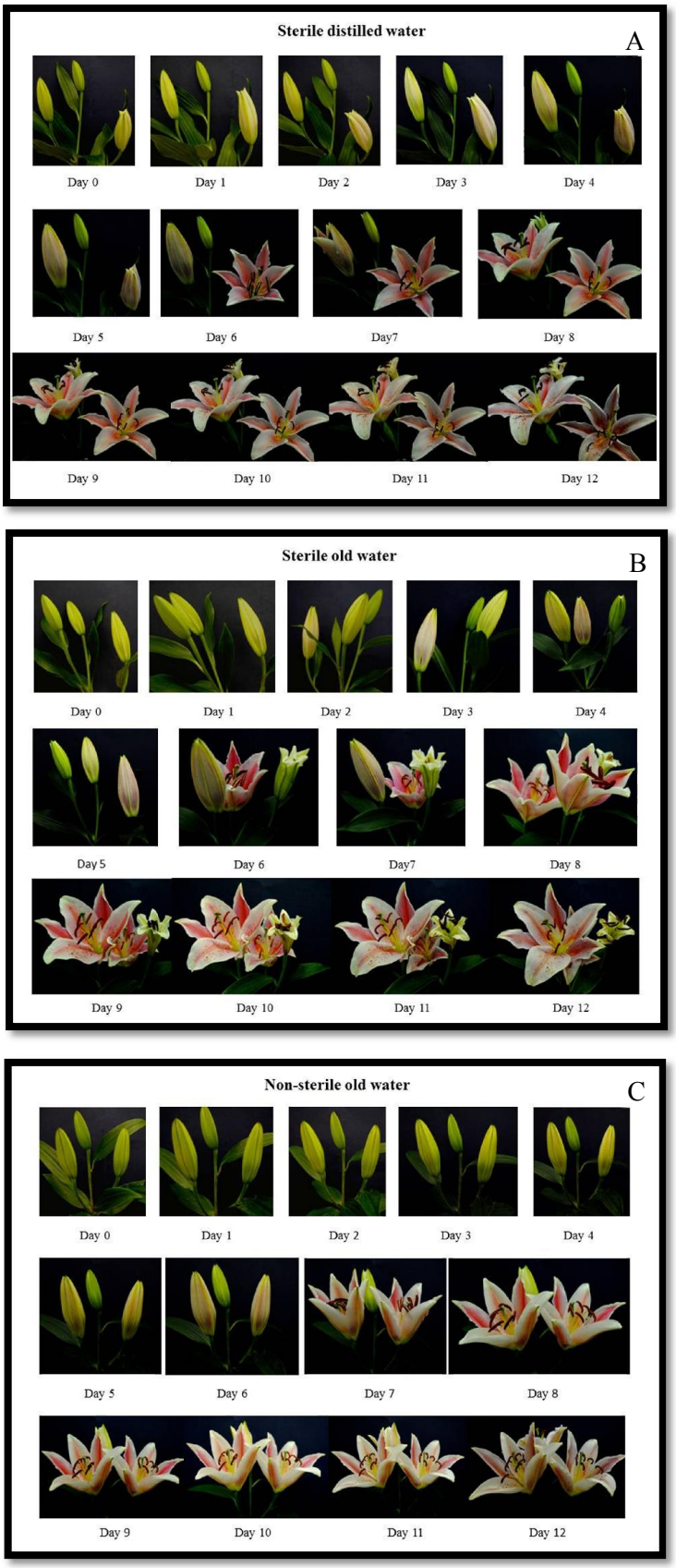


Figure 4.3: Overall appearance changes of the 'Tiber' lily held in sterile distilled water (A), sterile old water (B) and non-sterile old water (C) during the 12 days of vase life.

The total bacterial plate count in the vase water of all treatments was evaluated at day 0 and day 12. Bacteria were found only in non-sterile old water while in sterile distilled water and sterile old water they were not detected at day 0. Significant populations of bacteria were found in all treatments at day 12. However, bacteria in the non-sterile old water had a significantly greater number than those in sterile distilled water and sterile old water (Figure 4.4).

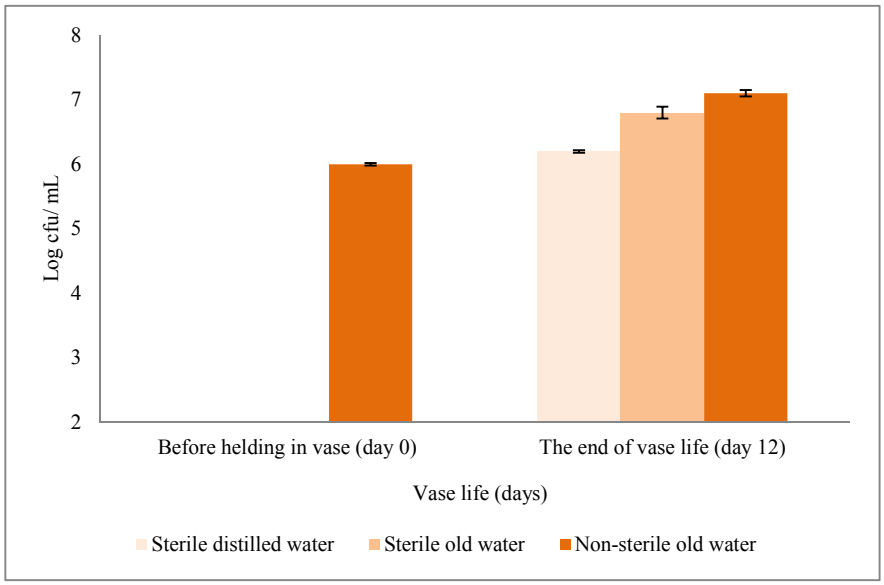


Figure 4.4: Changes of total bacterial plate count of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water during the 12 days of vase life. Data are means of three replications, ± SD.

4.3.2 Investigation into chemical exudates from 'Tiber' lilies stems on the vase life of 'Akito' rose

The vase water that had previously been used to hold two stems of 'Tiber' lily until the end of vase life was use to investigate the effect of chemical exudates on the vase life of the 'Akito' rose.

Changes of water uptake rate of 'Akito' roses held in sterile distilled water, sterile and non-sterile old water were compared throughout the vase life. The water

uptake of ‘Akito’ roses of all treatments increased during the first three days and then slightly decreased until the end of the vase life. However, the water uptake of the ‘Akito’ roses held in all the waters was not significantly different (Figure 4.5).

Changes in the fresh weight of the ‘Akito’ roses increased in the first stage and slightly declined throughout the vase life. The fresh weight of the ‘Akito’ roses held in sterile distilled water increased significantly more than those held in sterile and non-sterile old water at day 1 and day 3. However, the fresh weight of ‘Akito’ roses held in all waters were not different after day 3 (Figure 4.6).

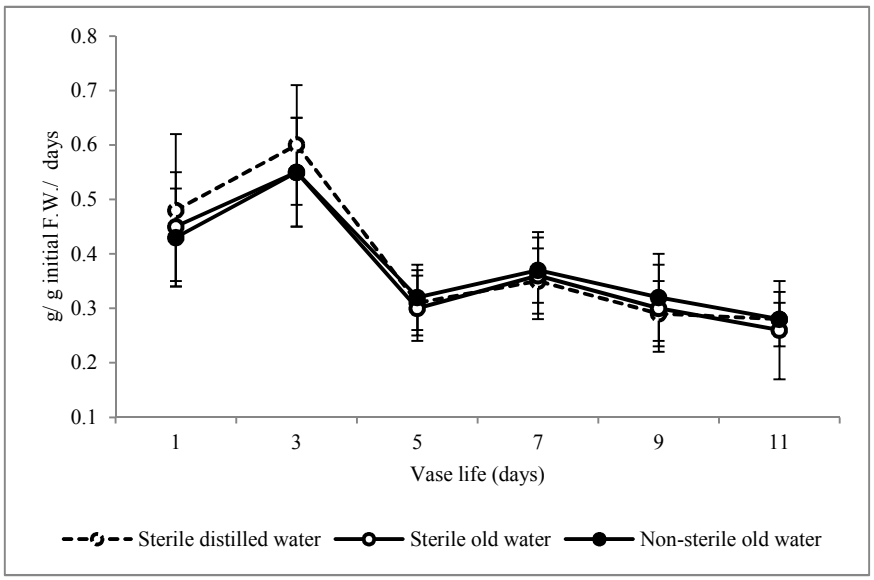


Figure 4.5: Changes of water uptake of the ‘Akito’ rose held in sterile distilled water, sterile old water and non-sterile old water during the 12 days of vase life. Data are means of three replications, ± SD.

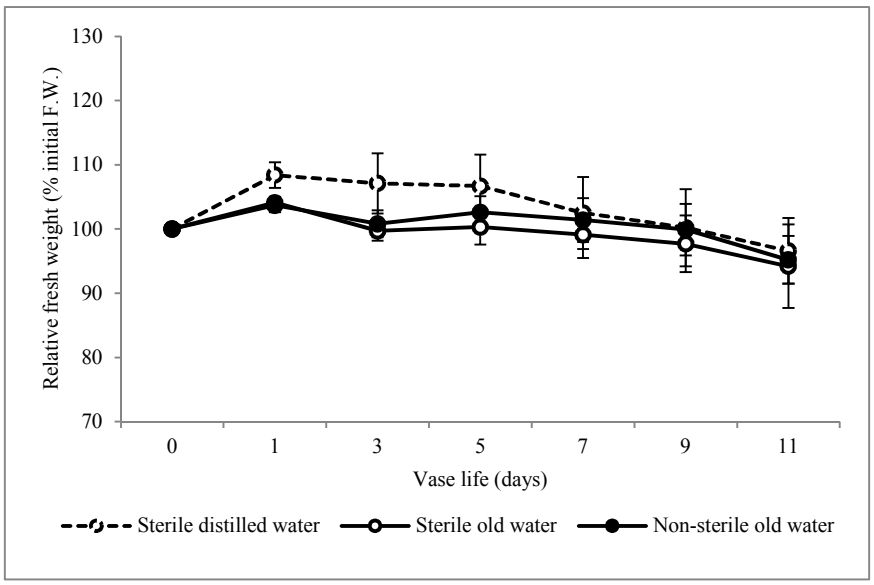


Figure 4.6: Changes of the fresh weight of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water during the 12 days of vase life. Data are means of three replications, ± SD.

Table 4.2: Final stage of bud opening and vase life of the 'Akito' rose.

Treatment	Final stage of bud opening (days)	Vase life (days)
Sterile distilled water	4.5	9.5
Sterile old water	4.2	9.4
Non-sterile old water	3.7	9.0

The final stage of bud opening of the flowers held in sterile distilled water, sterile and non-sterile old water were 4.5, 4.2 and 3.7, respectively (Table 4.2). Data from the final stage of bud opening were not significantly different. The vase lives of 'Akito' roses held in sterile distilled water, sterile old water and non-sterile old water were 9.5, 9.4 and 9.0 days, respectively. However, data from the vase lives were not significantly different (Table 4.2).

The overall appearance changes of the ‘Akito’ roses held in distilled water, sterile and non-sterile old water is show in Figure 4.7. There were differences in characteristics of opening. The ‘Akito’ roses held in non-sterile old water treatment expressed bud opening differently to other treatments other treatments.

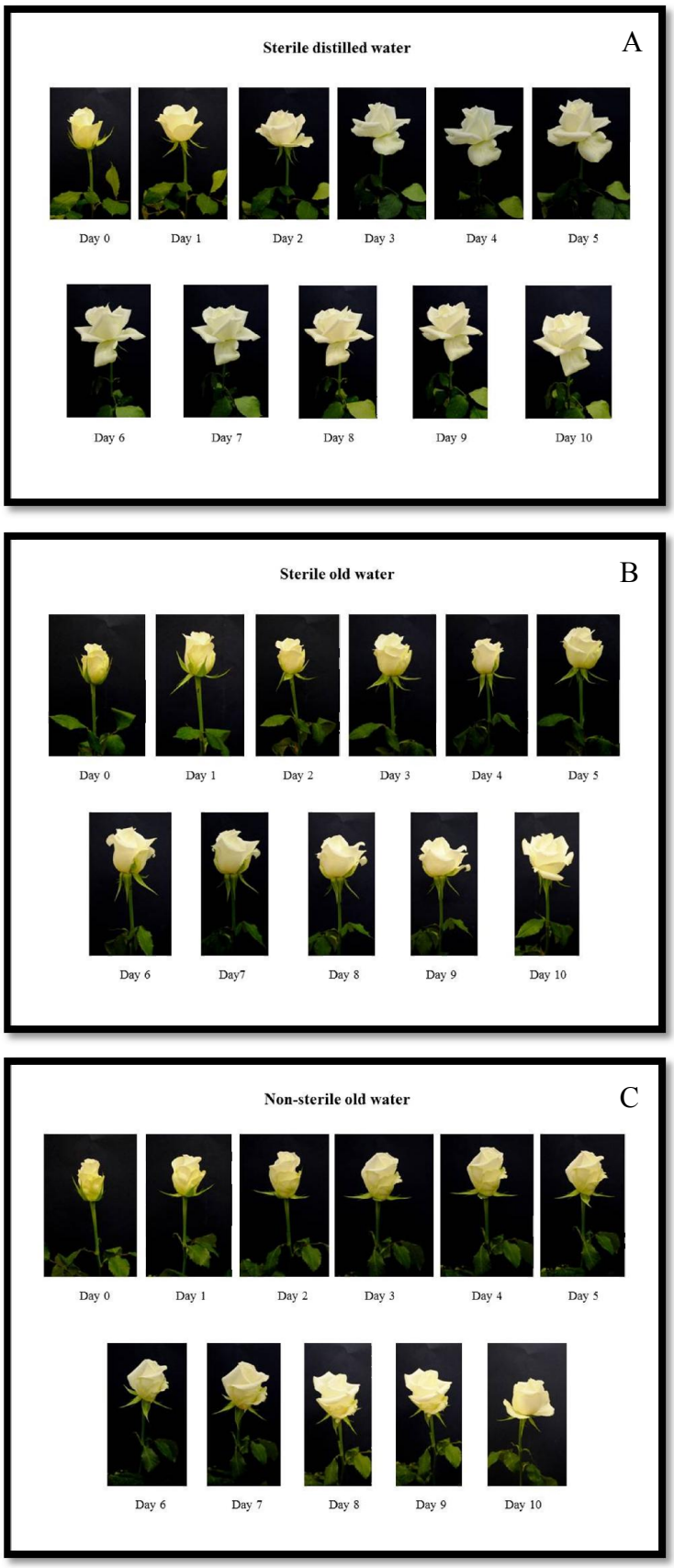


Figure 4.7: Overall changes in appearance of the 'Akito' rose held in sterile distilled water (A), sterile old water (B) and non-sterile old water (C) during the 12 days of vase life.

The bacteria plate count in the vase water of all treatments was evaluated at day 0 and day 10. Bacteria were found only in non-sterile old water at day 0. However, significant populations of bacteria were found in all treatments at day 10. Numbers of bacteria in non-sterile old water were more than those sterile distilled water and sterile old water. However, data were not significantly different (Figure 4.8).

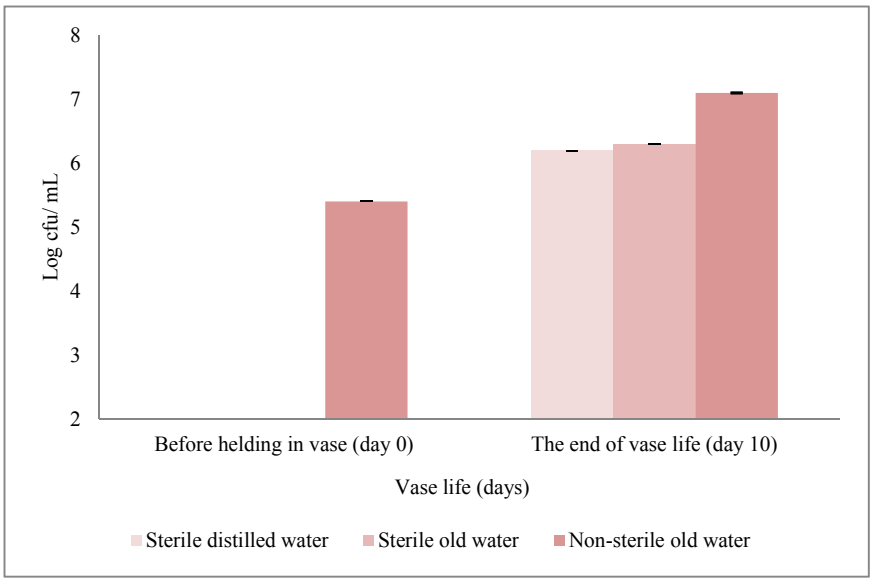


Figure 4.8: Changes in the bacteria plate count of the 'Akito' rose held in sterile distilled water, sterile old water and non-sterile old water during the 12 days of vase life. Data are means of three replications, ± SD.

4.4 Discussion

Previous research suggested that the quality of water is important for the maintenance of cut flowers. The stems of some plants exude sap and this may consist of cations, anions, amino and organic acids or chemical-like phenols that can give harmful toxins to other cut flowers or indeed to themselves (van Meeteren, 2000; Teixeira da Silva, 2003). Therefore, this experiment intended to study the role of chemical exudates on the vase life of the 'Tiber' lily and 'Akito' rose. The study was separated for observations of 'Tiber' lily and 'Akito' rose.

For the ‘Tiber’ lily, the old water seemed to have an effect on water uptake and gain of fresh weight during first three days. Both sterile and non-sterile old water treatments had water uptake and gains of fresh weight less than those of the sterile distilled water. However, these parameters were not different after day three. Consideration of overall appearance, time to open of the primary bud, and vase life found that there were no differences among treatments.

However, data for bacterial plate counts at the end of vase life showed that there was an increase in the number of bacteria in the vase water of all treatments. Small numbers of bacteria that grew in the vase water may have come from flower stems even though all stems were cleaned with DICA (Sodium dichloroisocyanuric acid) before treatment. Therefore vase life and other characteristics noted in these experiments may have been primarily influenced by the development of the bacterial populations.

For the ‘Akito’ roses, the chemical exudates also had an effect on their fresh weight during first three days. Both sterile and non-sterile old water treatments significantly gained fresh weight less than with sterile distilled water. However, all treatments were not different after day three. Moreover, the final stage of bud opening, and vase life of all treatment were not different. However, the overall appearance of non-sterile old water treatment in terms of bud opening was not completely the same as the other treatments. This may be due to the high number of bacteria in the vase water at the beginning stage.

Data from the bacterial plate count at the end of the vase life showed that there was an increase in the number of bacteria in the vase water of all treatments. As for the ‘Tiber’ lily, the bacterial populations may have had a dominant effect on properties such as vase life in these experiments.

These experiments showed differences in water uptake and fresh weight only during the first three days of vase life in both the ‘Tiber’ lilies and ‘Akito’ roses. After that, any significant differences disappeared, which may have been due to the influence of the developing bacterial populations. In spite of this, previous studies into the effects of water quality have found that the chemical composition of the water was a factor that had an effect on a number of parameters; for example, the bud opening of cut carnations (Rogers, 1973). Moreover, chemical compounds were shown to be

released from stems and leaves into the vase water causing a breakdown of stem cells and inducing a blockage in the xylem by degradation products. Moreover, sugars, proteins and polyphenols have been shown to have a harmful effect on the vase life of cut roses (Zieslin, 1989). However, as discussed in Section 4.2, exudates can, in some instances, have a beneficial effect on flower characteristics.

The effect of chemical exudates on flowers was not clear in the present study as they only had an effect during the early part of the vase life. The vase lives of 'Tiber' lilies and 'Akito' roses in all treatments were not significantly different because the bacterial population increased later after the flowers had been held in the vase. Even when all flowers were cleaned with 50 ppm DICA (Sodium dichloroisocyanuric acid) before being held in the water, some bacteria appeared to colonise and grow in the water. The increase of bacteria may have been the most important factor limiting the vase life of flowers. The contamination by bacteria may come from bacteria that accumulate in the xylem (van Doorn, 1995). Such bacteria would be difficult to remove with the chemical treatment used on the stems in this study.

4.5 Conclusions

An investigation into the role of chemical exudates on the vase life of the 'Tiber' lily and 'Akito' rose was separated into two experiments:

For the 'Tiber' lily, the influence of the old water seemed to have an effect on water uptake and their fresh weight during first three days of vase life. Chemical exudates did not have an effect on overall appearance, time to open of the primary bud, and vase life. Moreover, this experiment found an increase in the number of bacteria in the vase water of all treatments, so flower characteristics, such as vase life, may have been due primarily to the development of bacterial populations.

For the 'Akito' rose, there were also treatment effects on fresh weight during the first three days. However, treatment effects for final stage of bud opening, and vase life of all treatments were not significantly different. This experiment also found an increase in the number of bacteria in the vase water of all treatments, as found for

the ‘Tiber’ lily. Therefore, as for the ‘Tiber’ lily, flower characteristics, including vase life, may have been mainly due to the presence of bacteria.

The role of chemical exudates on flowers was not clear in the present study as they only had an effect during the early part of the vase life. The vase lives of ‘Tiber’ lilies and ‘Akito’ roses in all treatments were not significantly different. Perhaps treatment effects caused by the presence of chemical exudates may have been ‘masked’ by the presence of micro-organisms.

CHAPTER FIVE

Consideration of the usefulness of essential oils and weak acids for the control of microbial growth in vase water

5.1 Introduction

Increases in bacterial populations in vase water may have an important effect on vase life of cut flowers, as previous work in the current research has suggested. Improving the water balance in cut flowers by using chemical compounds has been studied for many years. Silver nitrate and silver thiosulphate are very popular in the cut flower industry. Today, the use of silver compounds for cut flowers has been reduced because of their possible effect on human health and their environmental risk. However, the new nanoparticle compounds such as nanometer-sized silver (Ag^{+}) particles (NS) are effective in reducing bacterial populations and extending the vase life of cut gerbera flowers (Liu *et al*., 2009). Pre-treatment (Pulsing) the 'Movie Star' rose with Nano-silver could reduce decreases in fresh weight and extend vase life (Lu *et al*., 2010). The other compounds used as antimicrobial agents are sodium hypochlorite (NaOCl), hydroxyquinoline compounds and quaternary ammonium compounds, and some of these are present in commercial 'flower food' But all of these compounds (including nanosilver) may have an important environmental impact, and could be subject to increased regulation in the future

At present, consumers are concerned about the environment, both the conditions under which flowers are produced and also how they are displayed (CBI market survey, 2007). This concern extends to the chemicals used during display. Therefore, developing new substances as alternatives to these compounds currently used by the floriculture industry could be important. Essential oils are one of the groups of natural products which could be of interest to the industry due to their effects on microorganisms and also because they are generally safe for humans and the environment. The antimicrobial activity of essentials oils has been studied in relation to many applications. For example, they were applied in fruit and vegetable washing water. The results showed successful results against natural spoilage flora and food-borne pathogens (Burt, 2004). In fact, essential oils are used for many

reasons, such as prolonging shelf-life in food products, enhancing the stability of fats and oils with high polyunsaturated fatty acids, and delaying ageing processes etc. (Baratta *et al*., 1998). One additional benefit for using essential oils as a 'preservative' in flower vase water is that it could also serve as a room 'fragrancer' or 'air freshener', which could make their use more desirable.

The common practice for extending vase life, apart from using specific antimicrobial compounds, is to use citric acid for adjusting the pH in vase water down to 3-4. Citric acid is a main ingredient in the vase solution. Low pH conditions reduce the increase of bacterial population and improve the water balance in xylem vessels of cut flowers (Darendeh and Hadavi, 2012). Vase life and quality of cut flowers are improved by using organic acids and carbohydrates (e.g. sucrose) (Sabzi *et al*., 2012).

At present, the food industry uses weak organic acids as a type of food preservative in food and beverages products (e.g. bakery products commonly use proprionic acid in the form of calcium proprionate). However many of these compounds have not previously been investigated for their ability to control bacterial growth in vase water. Their main advantage in terms of consumer use is that they are safe, 'food grade' materials.

The objective of this experiment was to investigate an alternative way for controlling bacteria in vase water. This study was divided into two topics. First was the consideration of essential oils as antimicrobials for reducing bacterial populations in cut flowers. The second was an investigation of the preliminary effect of a range of weak organic acids, some of which are also used as a food preservative, on bacterial growth for cut flowers

5.2 Materials and methods

The first experiment was a study on the effect of essential oils for controlling bacteria in the vase water of the 'Tiber' lily and 'Akito' rose. The second study used a screening test with weak organic acids on six bacterial strains.

5.2.1 The effects of essential oils on bacterial growth in cut 'Tiber' lily and 'Akito' rose

5.2.1.2 Screening of essential oils

Bacteria were isolated from the vase water from the five experiments described in Chapter 3 (i.e. L4, L7, L9, L15, L29, A1, A36, A55, V1, V20, V45, LA15, LA18, LA45, LAF8, LAF10, LAF13, and LAF18) and were tested with various essential oils. The bacteria were grown overnight in flasks containing 100 mL TSB (Tryptone Soya Broth, Oxoid) at 37°C for 24 h. Then each inoculum was diluted into a concentration of 10^5-10^7 cell/ mL through the use of a calibration standard produced from investigation of the O.D. and plate count number, followed by inoculation on the surface of TSA (Tryptone Soya Agar, Oxoid).

Twelve pure essential oils were selected for this study: West Indian bay, cinnamon leaf, clove bud, clove leaf, Chinese ginger, lemongrass, mandarin, rosemary, sage, spearmint, sweet fennel, and thyme, (F.D. Copeland and Sons Ltd, London)) These were all diluted 1:10 with methanol to produce a stock solution of 100mg/ml of the pure essential oil. A disc diffusion technique was used to screen the effective essential oils. Sterile filter paper discs (6 mm in diameter) were dipped in each treatment and were placed on the surface of each inoculated plate. On each plate were placed three discs of one essential oil and other one was methanol as a control (Cairns and Magan). The plates were incubated at 37°C for 48 h. A zone of inhibition with halos equal to or greater than 7 mm was considered to be an effective essential oil (Nascimento *et al*., 2000).

The essential oils shown as being the most effective against bacteria were then tested to establish the minimum bactericidal concentration (MBC) for each bacterium.

5.2.1.3 Minimum Bactericidal Concentration (MBC)

Twelve glass test tubes with screw caps (16x150 mm) were filled with 1 mL of TSB except for tube No. 1, and then all tubes were sterilized. For tube No.1 and No. 2 of the series, 1 mL of essential oil stock solution was added. Tube No. 2 was stirred and 1 mL was withdrawn and transferred to tube No. 3. These continued 1:1 dilutions were repeated until tube No. 12. Finally, 1 mL of inoculum was added to all tubes. Due to essential oil cannot dissolve in water, the stock solution of essential oil was prepared in 5% w/v glycerol in water. The suspension of essential oil was diluted from 50 mg/ mL to 0 mg/ mL and the tubes were incubated at 37°C for 48 h. The

MBC is defined as the lowest concentration at which there is growth of bacteria on TSA (Mazzola *et al*., 2009; Rangrianarivelo *et al*., 2009).

5.2.1.4 The effect of thyme oil on the vase life of the 'Tiber' lily and 'Akito' rose

(a) Plant material

'Akito' roses and 'Tiber' lilies at the commercial sold stage were obtained from Flamingo Holdings Company Ltd., (Gt North Road, Sandy, Bedfordshire SG19 2AJ). All the flowers were transported to the Microbiology Laboratory at Cranfield University, UK. After that, they were selected for uniformity and without defects. Stem lengths of all samples were re-cut to 50 cm under water using a sterile razor blade (van Doorn, 1997). Leaves on the lower one-third of stems were stripped. Within each stem of the lilies, the number of buds per stem was kept as constant as possible for determining the stem's vase life. A plastic film was used to cover the top of the vases to avoid water loss (Mayark *et al*., 1973).

(b) Experiment design

Experiment 1: Two stems of 'Tiber' lilies were stood in a 2-Litre vase containing 1,000 ml vase solution as followed;

- distilled water
- 5% w/v glycerol
- Chrysal (Commercial liquid flower food)
- 0.78 mg/mL thyme oil in 5% glycerol.
- 1.56 mg/mL thyme oil in 5% glycerol.
- 3.12 mg/mL thyme oil in 5% glycerol.
- 6.25 mg/mL thyme oil in 5% glycerol.
- 12.5 mg/mL thyme oil in 5% glycerol.
- 25 mg/mL thyme oil in 5% glycerol.

Experiment 2: Five stems of cut 'Akito' roses were stood in a 2-Litre vase containing 1,000 ml vase solution as followed;

- distilled water
- 5% w/v glycerol
- Chrysal (Commercial liquid flower food)
- 0.78 mg/mL thyme oil in 5% glycerol.
- 1.56 mg/mL thyme oil in 5% glycerol.
- 3.12 mg/mL thyme oil in 5% glycerol.
- 6.25 mg/mL thyme oil in 5% glycerol.
- 12.5 mg/mL thyme oil in 5% glycerol.
- 25 mg/mL thyme oil in 5% glycerol.

These experiments used distilled water, 5% glycerol and Chrysal as a control. The thyme oil at different concentrations was dissolved in 5% w/v glycerol before being applied to the flowers. For apply with cut flowers, the concentration was varied above and lower than MBC.

The experiment was a completely randomised design (CRD) with three replications. The vases of flowers were placed in the vase life room at 20°C and given a 12 hours on-off of cycle under a lamp throughout the vase life.

(c) Preparation of samples for microorganism estimation

Vase water from three replicates per treatment was sampled after stirring and 3 ml was aseptically pipetted into sterile bottles. Samples from the vases were taken aseptically at the time before the flowers were placed in the vase and at the end of the vase life. Protection against contamination was achieved by sealing a plastic film on the top of each vase.

One mL of vase water were diluted into 10 to 1,000-fold dilutions, and 100 µL of vase water were spread on sterile Tryptone Soya Agar (Oxoid), inoculated plates were incubated at 25°C for two days before being counted.

(d) Vase life

The cut flower longevity was recorded as days of vase life from the time the flowers were placed into the vases (day 0). The end of the vase life was indicated

when a score of flower quality reached stage 5 (refer to flower standard criteria in chapter 2).

(e) Fresh weight and water uptake measurements

The stem fresh weight (f.w.) and the vase weight (vase + water) were measured at the same time every day from day 0. These data were used to determine the relative fresh weight (RFW) as a percentage of the initial fresh weight (% initial f.w.) and water uptake as a g/ g initial f.w. per day.

(f) The pH of vase water

The pH of vase water was measured during the experiment using a pH meter (HANNA Instruments, Made in Portugal).

5.2.2 Investigation of using weak organic acids combined with a pH buffer in the control of bacteria in the vase water

Bacteria previously found in vase water of 'Tiber' lily and 'Akito' rose were selected for study in this experiment. Six species of bacteria (*Staphylococcus spp.* (L7), *Streptococcus spp.* or *Enterococcus* (A36), *Bacillus spp*. (L8), *Pseudomonas spp.* (L29), *Enterobacteria* spp. (L4), *Neisseria spp.* (A1)) were tested with various weak acids.

The bacteria were grown overnight in flasks containing 100 mL TSB (Tryptone Soya Broth, Oxoid) at 37°C for 24 h. Each inoculum was diluted into 10^5-10^7 cell/ mL through the use of a calibration standard produced from investigation of O.D. and plate count number, then inoculated on the surface of TSA (Tryptone Soya Agar, Oxoid).

The experiment was divided into two groups as followed;

- Screen of weak organic acids on TSA (pH 7 using distilled water)
- Screen of weak organic acids on TSA (pH 4 using citric acid phosphate buffer). This was carried out because the performance of the weak acids is likely to be optimal under these conditions.

Weak organic acids used were as follows: adipic acid (99%, Sigma Aldrich), benzoic acid (99%, Acros Organics), Trans-cinnamic acid (Aldrich), ferulic acid (HPLC Grade), fumalic acid (laboratory reagent grade, Fisher Scientific), gluconic acid lactone (Sigma Aldrich), propionic acid (99%, Acros Organics), sorbic acid (Sigma Aldrich) and tartaric acid (L(+)tartaric acid, Sigma Chemical Co.) were diluted into 1mg/ mL in distilled water except ferulic acid, which was diluted in absolute ethanol (HPLC Grade). The solutions were filter sterilized by use of a sterile 0.2 µm Millipore filter (Minisart, Sartorious) and dispensed into sterile bottles.

Sterile filter paper discs (6 mm in diameter) were dipped in each treatment and were placed on the surface of each inoculated plated. On each plate were placed three discs of one weak acid and distilled water was used as a control. The plates were incubated at 37°C for 48 h. The zone of inhibition with halos equal to or greater than 7 mm was considered to be an effective weak acid (Nascimento *et al*., 2000).

5.2.5 Statistical analysis

Significance tests were made by analysis of variance (ANOVA) using SPSS version 16. Mean comparisons were made using least significance difference (LSD).

5.3 Results

5.3.1 Effects of essential oils on bacteria growth of cut 'Tiber' lily and 'Akito' rose

5.3.1.1 Screening of essential oils

The effect of 12 essential oils was investigated on the 18 bacteria previously isolated from the vase water of cut flowers. The Disc diffusion assay method was used to compare the efficacy of essential oils. The results showed that thyme oil was most effective over other essential oils in the control of L4, A1, A36, A55, V20, V45, LA15, LA18, LAF8, LAF10, LAF13 and LAF18 (Fig 5.1). Moreover, lemongrass oil also were effective against A55, V1, V45, LA15, LA18, LAF8, LAF10, LAF13 and LAF18 (Fig 5.1). Other effective essential oils were west indian bay, cinnamon leaf, clove bud and clove leaf, but these were only effective against some

microorganisms (Figure 5.1). The first two essential oils (thyme and lemongrass) were effective against most bacteria and these were selected to determine MBC.

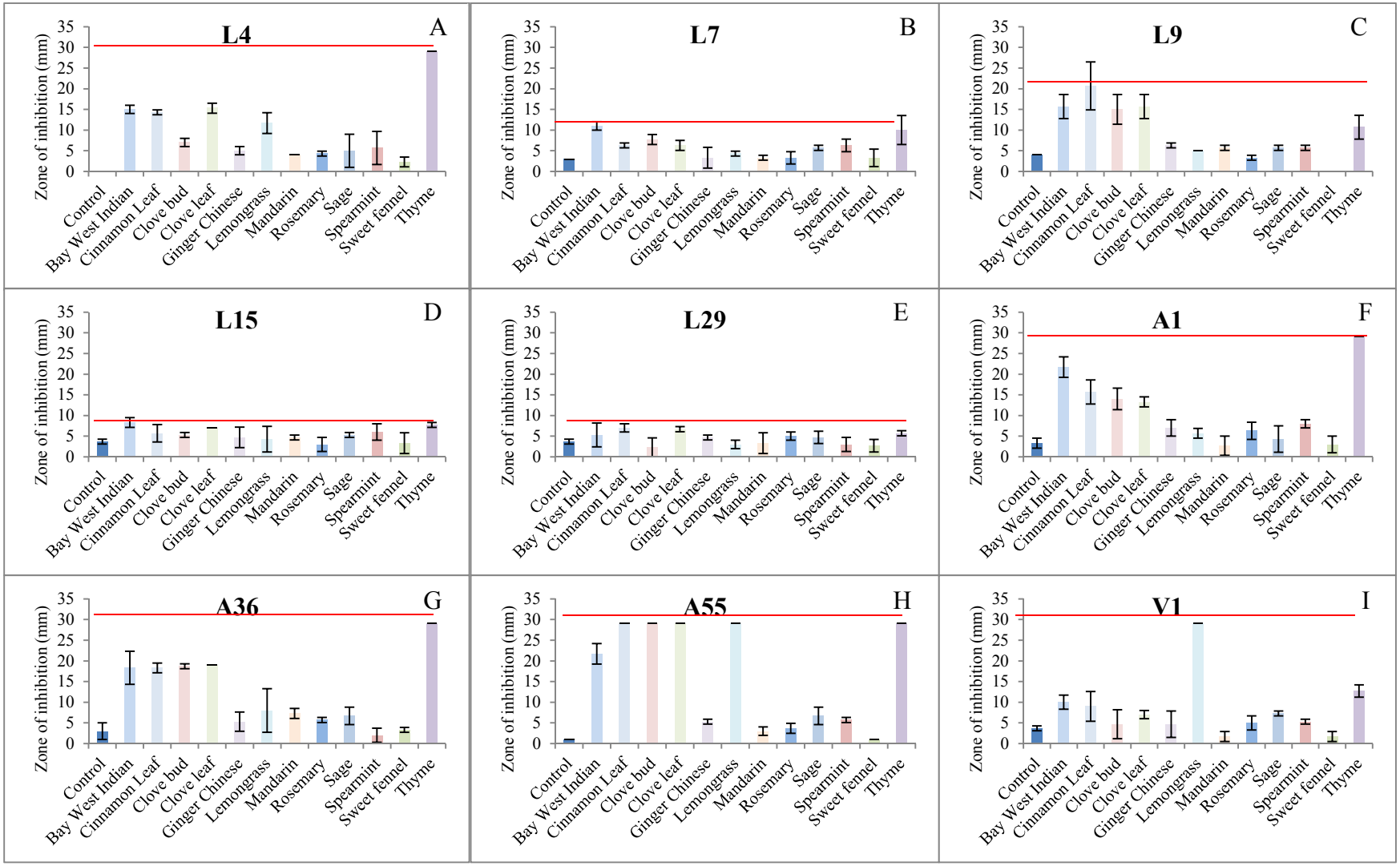


Figure 5.1: Zone of inhibition of microorganisms when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C; L4 (A), L7 (B), L8 (C), L15 (D), L29 (E), A1 (F), A36 (G), A55 (H), and V1 (I). Data are means of three replications, ± SD.

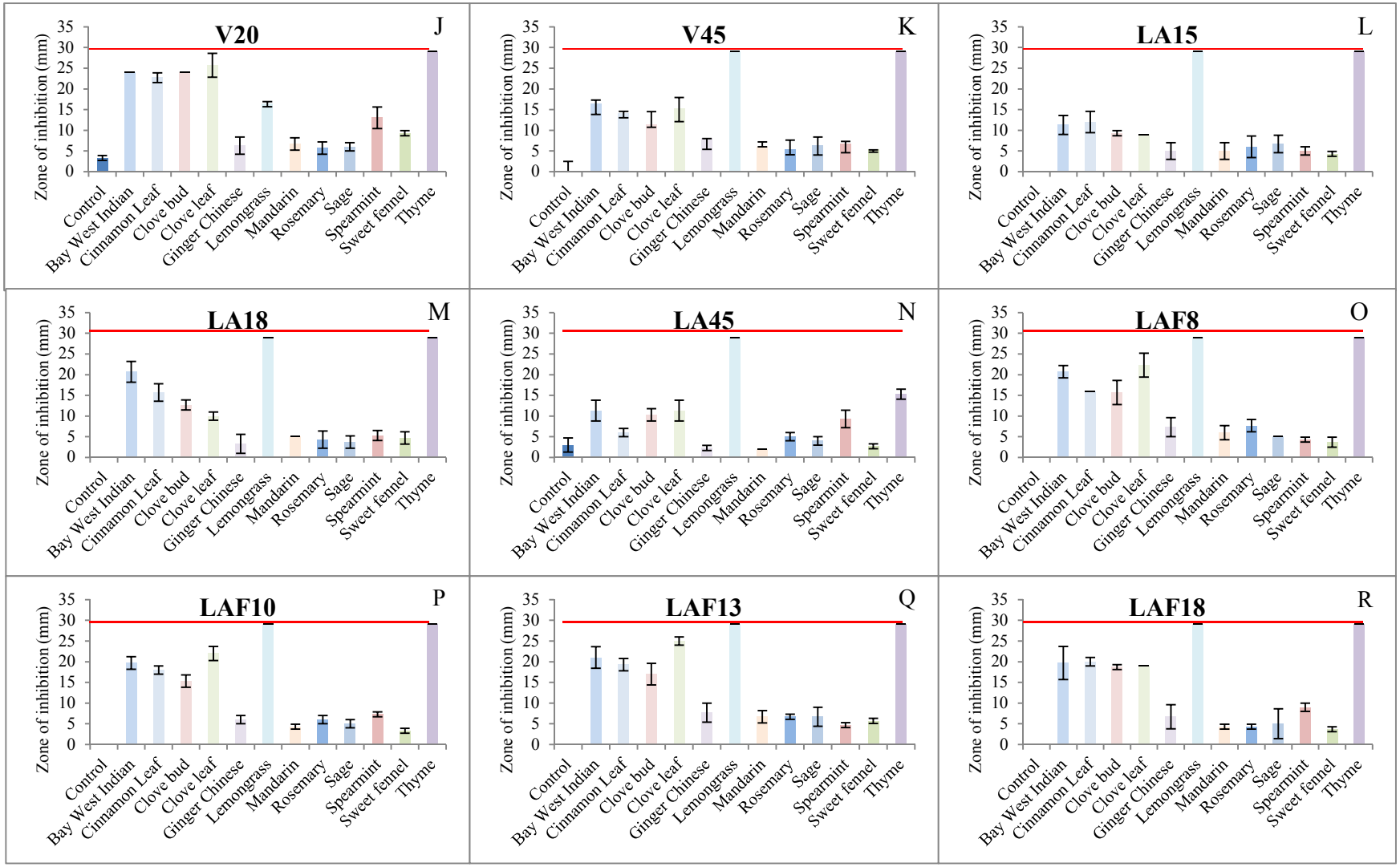


Figure 5.1 (cont.): Zone of inhibition of microorganisms when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C; V20 (J), V45 (K), LA15 (L), LA18 (M), LA45 (N), LAF8 (O), LAF10 (P), LAF13 (Q), and LAF18 (R). Data are means of three replications, ± SD.

5.3.1.2 Minimum Bactericidal Concentration

From the screening described in 5.4.1.1 above, thyme and lemongrass were effective against bacteria over other essential oils. Both of these were selected to determine the MBC.

Table 5.1: The MBC of thyme and lemongrass oil on the growth of 18 isolated bacteria after 48 hrs at 37^{o}C

Bacteria code	**MBC of thyme oil (mg/mL)**	**MBC of lemongrass oil (mg/mL)**
L4	12.5	6.25
L7	12.5	25
L9	12.5	50
L15	6.25	25
L29	12.5	50
A1	6.25	6.25
A36	6.25	12.5
A55	6.25	12.5
V1	6.25	12.5
V10	3.12	25
V45	25	25
LA15	25	50
LA18	25	25
LA45	25	25
LAF8	6.25	12.5
LAF10	12.5	50
LAF13	12.5	12.5
LAF18	12.5	12.5
Mean	**12.67**	**24.30**

The results of the MBC shown in table 5.1, indicated that thyme oil was more effective than lemongrass. The mean MBC for thyme oil was less than lemongrass. A mean of the MBC of thyme oil was 12.67 mg/ mL while the MBC of lemongrass oil

was 24.30 mg/ mL. From this result, thyme oil was selected to investigate the control of bacterial growth in the vase water of the 'Tiber' lily and 'Akito' rose.

5.3.1.3 The effect of thyme oil on the vase life of the 'Tiber' lily

Thyme oil as an antimicrobial agent was applied to the vase water of the 'Tiber' lily. The concentration of thyme oil was varied from 0.78-25.0 mg/ mL. The effect of the thyme oil was compared with distilled water and liquid flower food (Chrysal).

The bacteria plate count in the vase water was evaluated at days 0, 3, 6 and 9 (Figure 5.2). The results show that bacterial populations in vase water of all treatments except Chrysal increased throughout the vase life after day 0. Bacterial populations in the Chrysal treatment reduced from day 0 to day 3 and were not detected from day 6 to day 9.

For the thyme oil treatment, this had an effect against the bacteria only on day 0 to day 3. At day 0, the vase water of all treatments was collected after the flowers had been placed in the vase for 1 h. A bacteria plate count in the vase water with thyme oil at concentrations of 1.56 to 25 mg/mL was not detected. However, a bacteria plate count was detected in the treatment of distilled water, Chrysal, 5% glycerol and 0.78 mg/mL thyme oil, although the bacteria plate count in the Chrysal treatment was lower than in the distilled water, 5% glycerol and 0.78 mg/mL thyme oil.

At day 3, the treatment of thyme oil was shown to be effective against bacteria when the concentration was increased. Concentration of 6.25 to 25 mg/mL thyme oil had bacteria numbers significantly less than in the distilled water, 5% glycerol and other treatments of thyme oil. However, the bacterial population in the vase water of Chrysal treatment had the lowest numbers. During day 6 to day 9, the thyme oil treatment loss its effectiveness against the bacteria and was similar to the distilled water treatment. In fact the bacterial populations in the vase water of thyme oil treatments were more than in the distilled water, especially at day 9, while bacterial populations in the Chrysal treatment were not detected.

The vase water of all treatments were measured for pH at days 0, 3, 6 and 9 (Figure 5.3). The treatment with Chrysal had the lowest pH, i.e. around 3, throughout the vase life. The pH of thyme oil treatment and distilled water slightly increased from around 4 to 5 along the vase life.

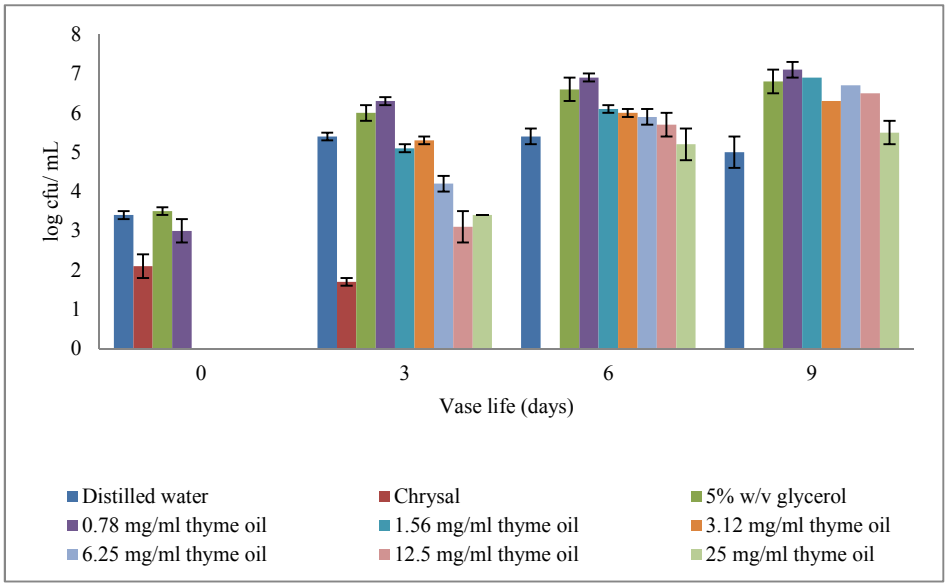


Figure 5.2: Bacteria plate count in the vase water of 'Tiber' lilies during the 9 days of vase life. Data are means of three replications, ± SD.

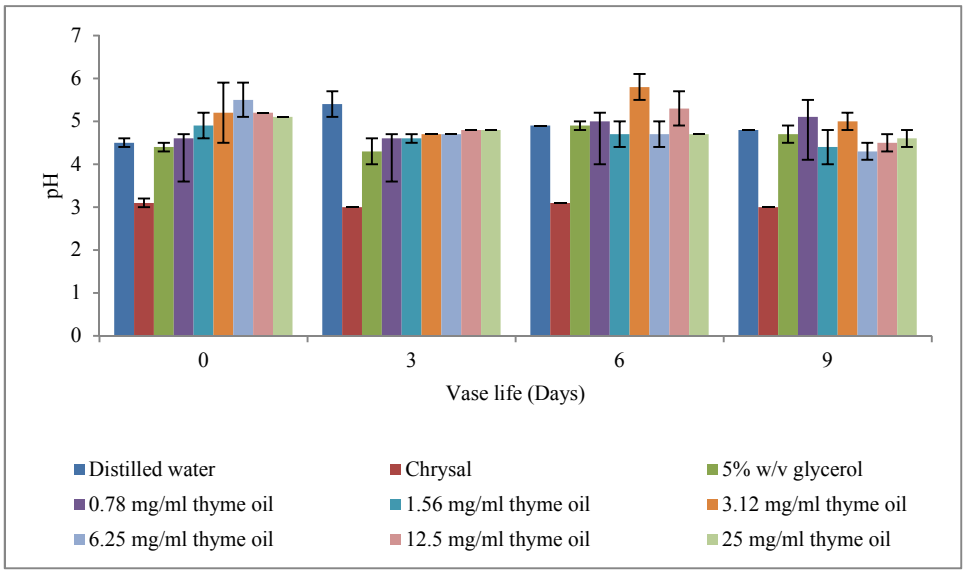


Figure 5.3: Changes of pH in the vase water of 'Tiber' lilies during the 9 days of vase life. Data are means of three replications, ± SD.

Changes of water uptake are shown in figure 5.4. Water uptake rates of all treatments were high at day 1 and started to decrease until day 5, then slightly increased again at day 7. At day 1, the water uptake of 'Tiber' lilies held in the Chrysal treatment was higher than other treatments followed by distilled water. The water uptake rates of flowers held in thyme oil at different concentrations were not significantly different. After day 1, the water uptake rate of the lily flowers held in distilled water was higher than other treatments, while the water uptake rate of lilies held in Chrysal, 5% glycerol and all thyme oil treatments were not significantly different during day 3 to day 5.

The fresh weight of 'Tiber' lilies in all treatments increased until day 3 or day 5 before they started to lose their weight throughout the rest of the vase life. A comparison of changes of fresh weight showed that the treatment in distilled water, Chrysal, 5% glycerol, 0.78 and 1.56 mg/mL thyme oil increased their fresh weight until day 5, while treatment of thyme oil at a concentration of 3.26 to 25 mg/mL increased their fresh weight until day 3. Lily flowers held in high concentrations of thyme oil lost their fresh weight faster than low concentrations and the control. The fresh weight of the lily flowers held in distilled water, Chrysal, 5% glycerol, 0.78 and 1.56 thyme oil were not significantly different (Figure 5.5).

The vase lives of 'Tiber' lilies held in different concentrations of thyme oil are shown in Figure 5.6. The results showed the vase life was shortened when the concentration of thyme oil was at its highest. The lowest concentration at 0.78 mg/ mL thyme oil gave the best results in increasing the vase life, as well as flower food (Chrysal), with 10.3 days. Concentration from 6.25 to 25 mg/ mL had the shortest vase life with 9 days. Interestingly, 'Tiber' lilies held in distilled water significantly had the longest vase life.

Overall, the appearance of 'Tiber' lilies at day 10 is shown in Figure 5.7. The primary bud of 'Tiber' lilies held in distilled water, Chrysal and 5% glycerol started to show signs of senescence such as sepals appearing with a little blueing symptom, while the primary buds of the 'Tiber' lilies held in thyme oil treatment appeared 'papery', and pale (unacceptable). Moreover, in the lilies held in thyme oil there was a blackening of stems while there was no blackening on the stems of lilies which were held in distilled water, Chrysal and 5% glycerol (Figure 5.8).

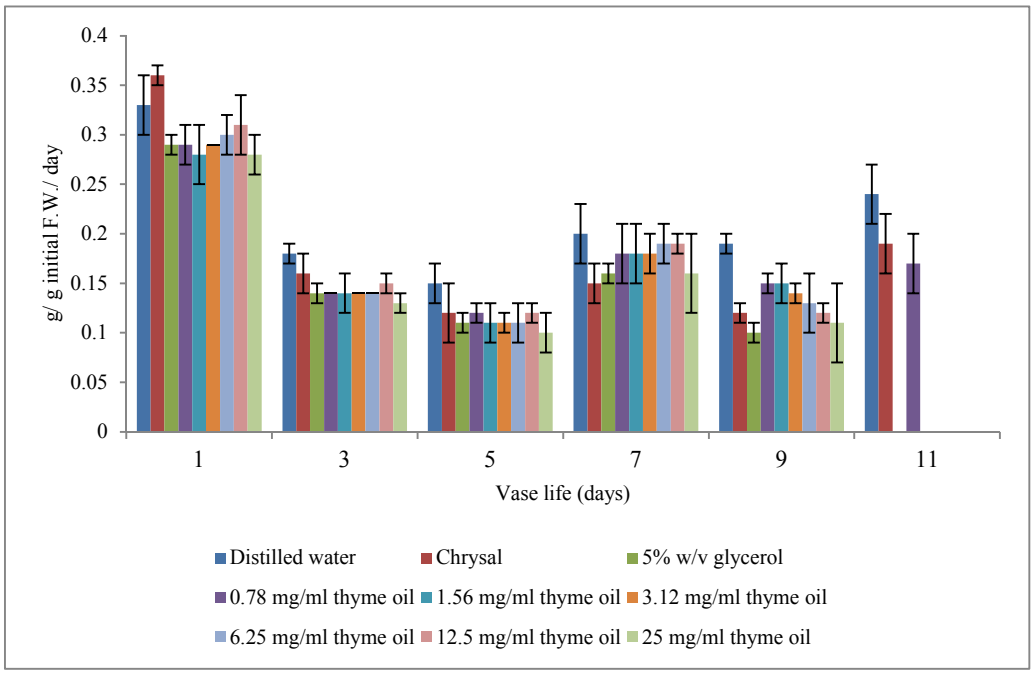


Figure 5.4: Changes in water uptake rate of the 'Tiber' lilies during the 11 days of vase life. Data are means of three replications, ± SD.

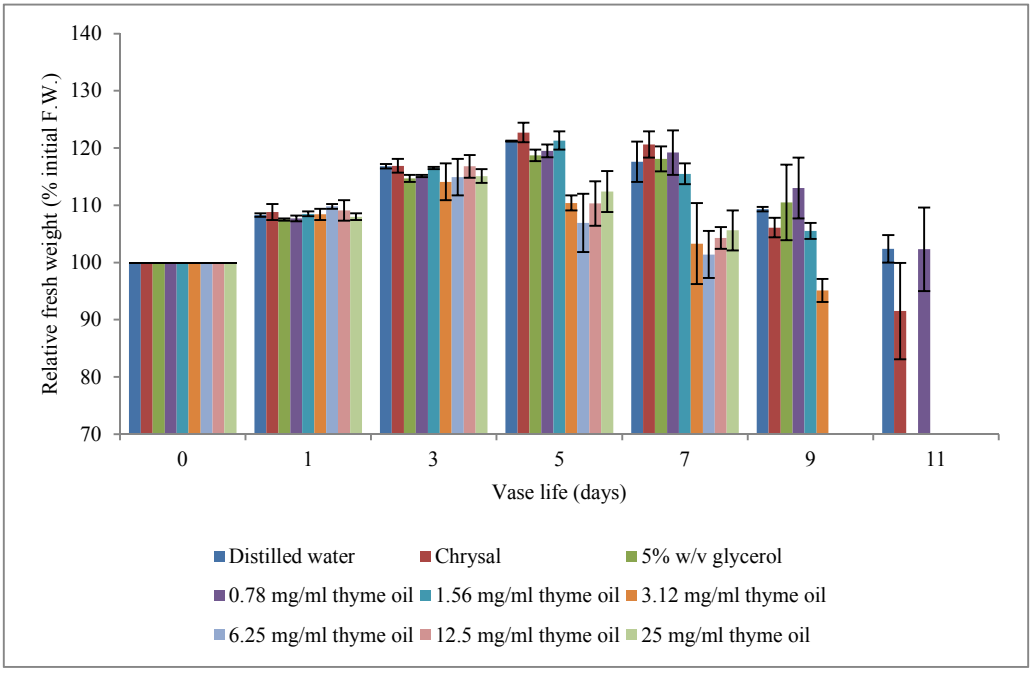


Figure 5.5: Changes of the fresh weight of the 'Tiber' lilies during the 11 days of vase life. Data are means of three replications, ± SD.

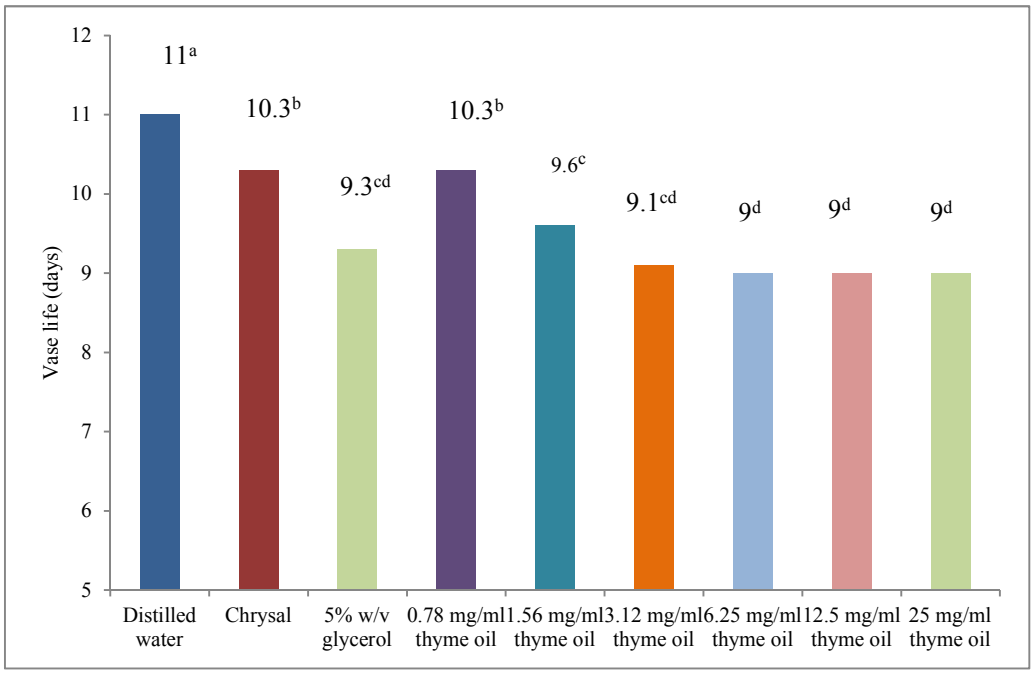


Figure 5.6: Vase lives of 'Tiber' lilies held in distilled water, Chrysal, 5% glycerol, 0.78 to 25 mg/ mL thyme oil. Means of the same main effect within the same letter(s) are not significantly different at P = 0.001 probability level.

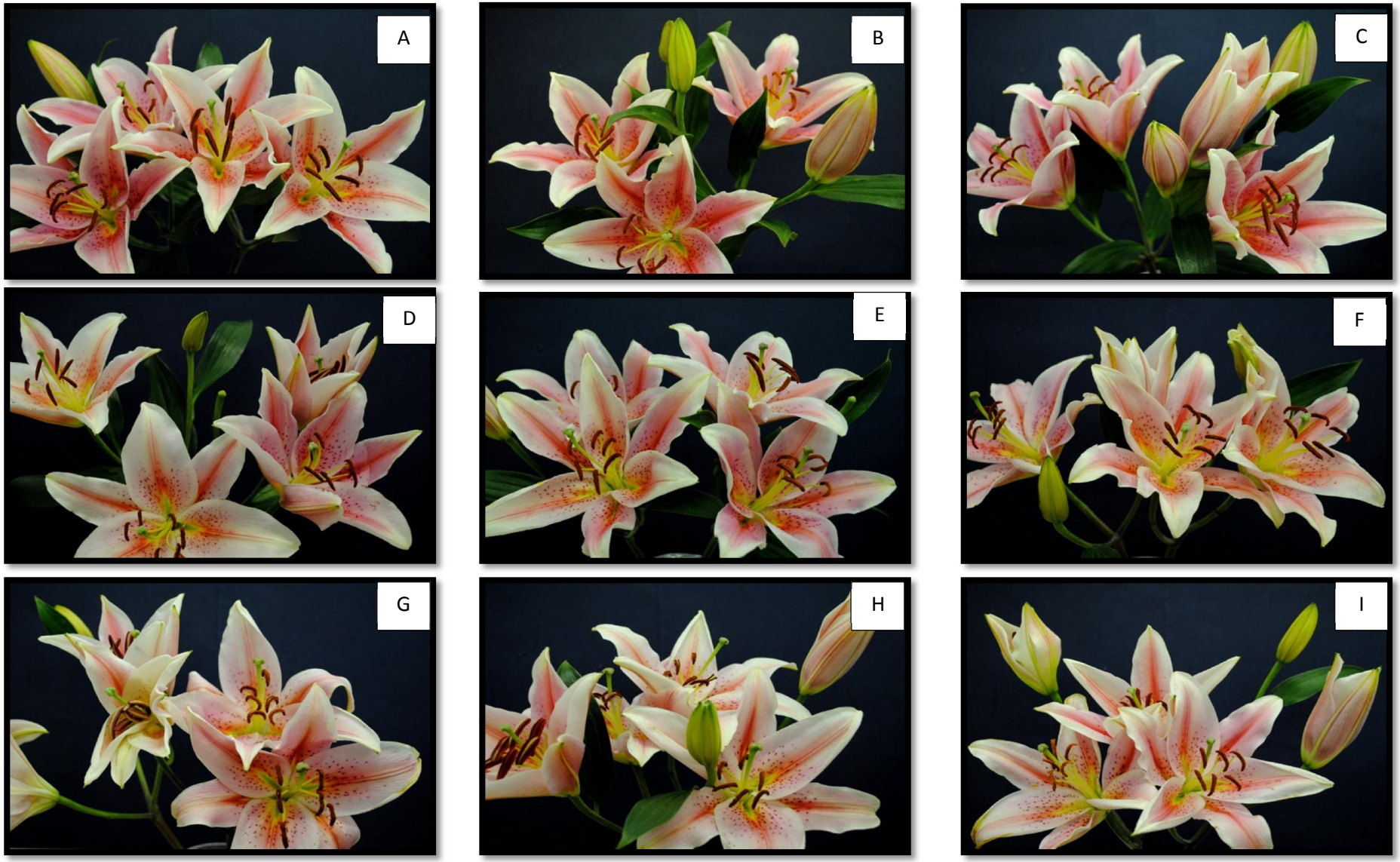
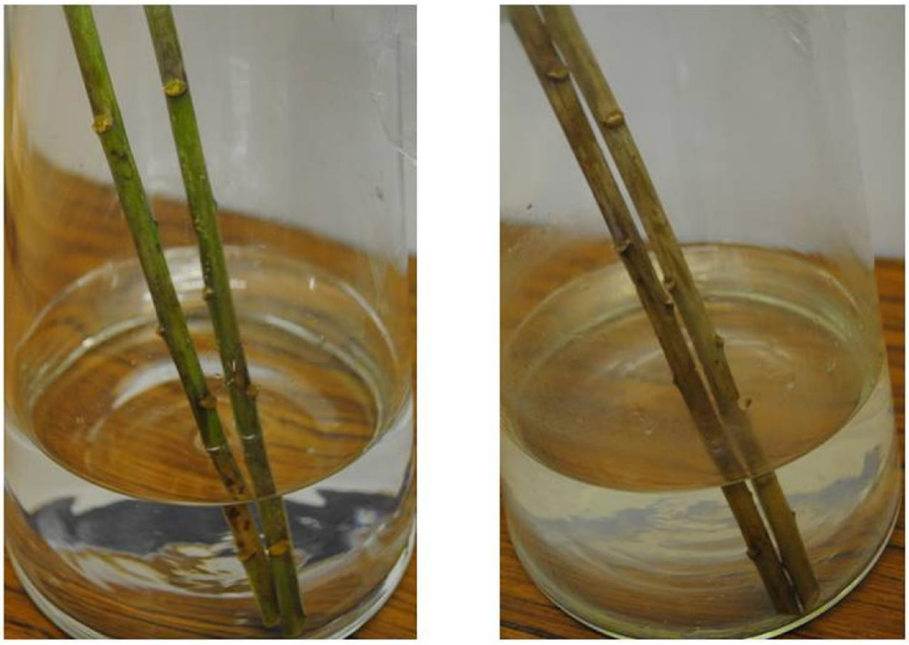


Figure 5.7: Overall appearances of the 'Tiber' lilies held in distilled water (A), Chrysal (B), 5% glycerol (C), 0.78 mg/ mL thyme oil (D), 1.56 mg/ mL thyme oil (E), 3.12 mg/ mL thyme oil (F), 6.25 mg/ mL thyme oil (G), 12.5 mg/ mL thyme oil (H), and 25.0 mg/ mL thyme oil (I), at day 10. (The primary buds of the 'Tiber' lilies held in thyme oil treatments appears papery and pale, there is an abscission of the top bud).



Stems held in distilled water

Stems held in Thyme oil treatment

Figure 5.8: Blackening symptom on stems of 'Tiber' lilies held in thyme oil treatment.

5.3.1.4 The effect of thyme oil on the vase life of the 'Akito' rose.

The effect of thyme oil on bacterial growth was investigated in the vase water of the 'Akito' rose. The concentration of thyme oil was divided into 0.78, 1.56, 3.12, 6.25, 12.5 and 25 mg/ mL. Distilled water, flower food (Chrysal), and 5% glycerol were used as controls.

The vase lives of 'Akito' roses held in different concentrations of thyme oil are shown in Figure 5.9. The results show that thyme oil could not extend the vase life of 'Akito' roses when compared with distilled water, Chrysal and 5% glycerol. The vase lives of roses held in thyme oil decreased with increasing concentration. The longest vase life was found in 'Akito' roses held in Chrysal, with 11.4 days while the vase life in distilled water and 5% glycerol were 5.7 and 6.1 days respectively. Vase life for thyme treatments of 0.78 to 25.0 mg/ mL thyme oil were 4.5, 3.2, 2.9, 2.8, 2.5 and 2.5 days, respectively.

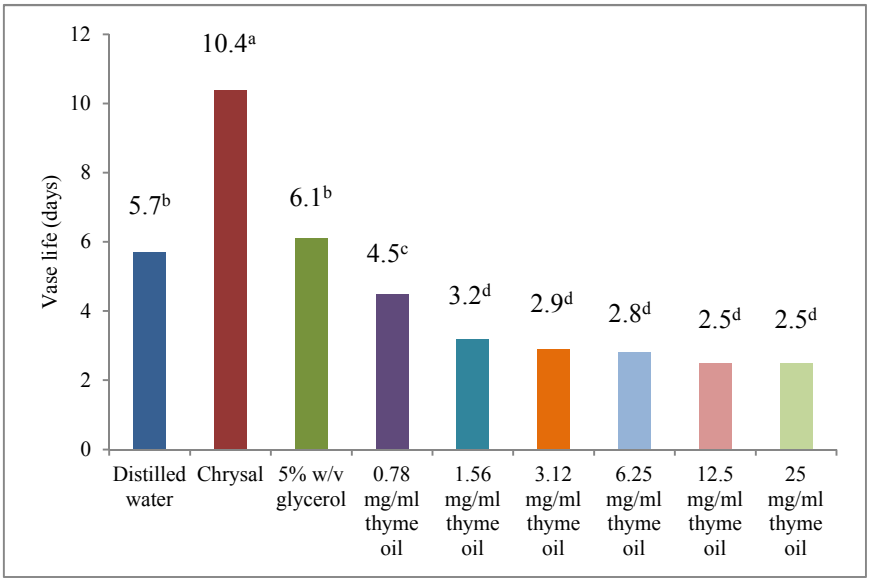


Figure 5.9: The vase lives of 'Akito' roses held in distilled water, Chrysal, 5% glycerol, 0.78 to 25 mg/ mL thyme oil. Means of the same main effect within the same letter(s) are not significantly different at P = 0.001 probability level.

Bacteria were detected in the vase water at days 0, 3, 6 and 9 (Figure 5.10). The results showed that the thyme oil treatment was effective against bacteria in vase water, over the treatment of distilled water and Chrysal, only on day 0. All concentrations of thyme oil treatments lost their effectiveness against bacteria at day 3. Bacterial population sizes for all concentrations of thyme oil and distilled water were not significantly different. Bacterial population size in the vase water with 5% glycerol was higher than with the other treatments. No bacteria were detected in vase water containing Chrysal after day 0. After day 3, the flower quality of 'Akito' roses held in 1.52 to 25 mg/ mL thyme oil were not acceptable.

There were only four treatments remaining at day 6, only the lowest concentration of thyme oil had not reached the end of vase life. The number of bacteria in the vase water with5% glycerol and 0.78 mg/ mL thyme oil were more than in the distilled water and Chrysal. Chrysal was clearly the best treatment for the control of bacteria growth.

Changes of pH in the vase water of all treatments, are shown in Figure 5.11. The Chrysal treatment had the lowest pH, at around 3, throughout the vase life. The pH of distilled water was around 5 at day 0, while the pH of the thyme oil in all treatments was around 4. However, the pH of the thyme oil treatments increased at day 3. The pH of thyme oil treatments was nearly 5 at the highest concentration.

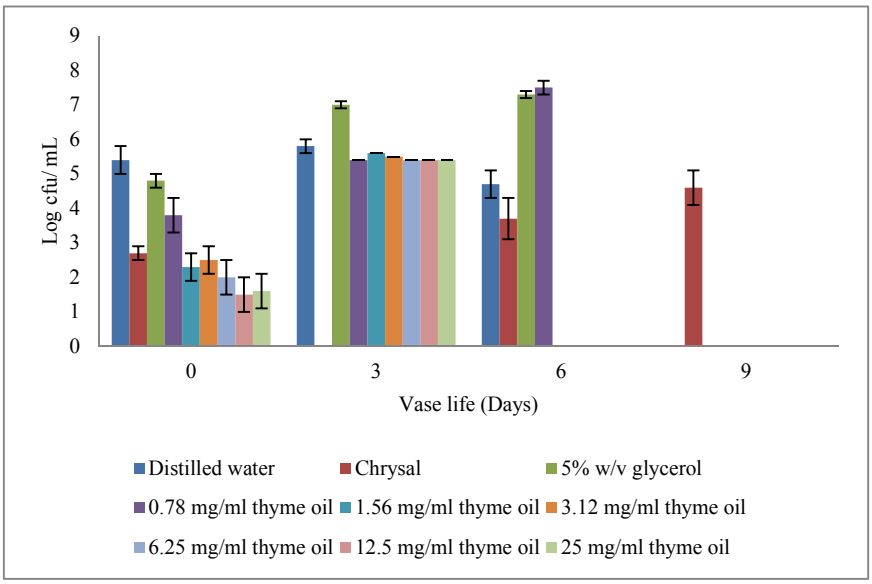


Figure 5.10: Total bacterial plate count in the vase water of the 'Akito' roses during the 9 days of vase life. Treatments of thyme oil were stopped from day 3 to day 9.Only data for 0.78 mg/ml data is shown for day 6. Distilled water and 5% w/w glycerol treatments were stopped from day 6 and data is not shown for day 9. Data are means of three replications, ± SD.

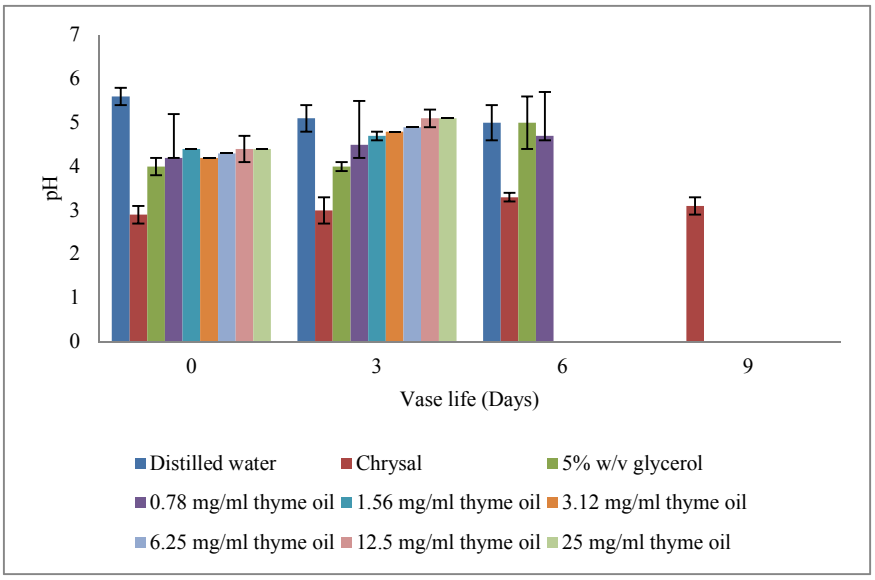


Figure 5.11: Changes in pH in the vase water of the 'Akito' roses during the 9 days of vase life. Treatments of thyme oil were stopped from day 3 to day 9.Only data for 0.78 mg/ml data is shown for day 6. Distilled water and 5% w/w glycerol treatments were stopped from day 6 and data is not shown for day 9. Data are means of three replications, ± SD.

Changes of water uptake are shown in figure 5.12. The water uptake rate of 'Akito' roses held in distilled water and Chrysal increased until day 3 and then slightly declined. In contrast, the water uptake rate of flowers held in 5% glycerol and all concentrations of thyme oil decreased throughout the vase life. The water uptake rates of roses were reduced with increasingly high concentrations of thyme oil, while the water uptake rate of roses held in Chrysal were higher than other treatments.

The fresh weight of 'Akito' roses of all treatments increased until day 2 and then slightly declined except for the Chrysal treatment. The fresh weight of flowers held in Chrysal increased until day 5 before declining for the remainder of vase life. However, the fresh weights of all treatments were not significantly different at day 1 and day 2. At day 3, the fresh weight of roses held in Chrysal still increased but other treatments started to lose their fresh weight. Fresh weights of 'Akito' roses held in

distilled water, 5% glycerol and 0.78 to 25 mg/ mL were not significantly different (Figure 5.13).

The overall appearance of 'Akito' roses at day 3 is shown in Figure 5.14. 'Akito' roses held in distilled water, Chrysal and 5% glycerol were still fresh and some of the buds had started to open. On the other hand, 'Akito' roses held in the thyme oil treatment showed signs of senescence. Moreover, stems of roses held in thyme oil showed a blackening while roses held in distilled water, Chrysal and 5% glycerol had no symptoms on their stems (Figure 5.15).

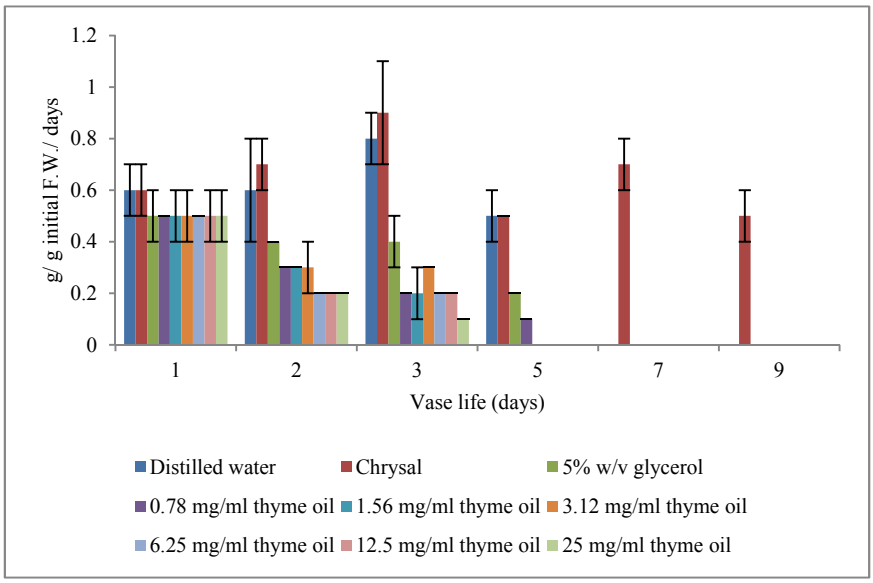


Figure 5.12: Changes in water uptake rate of the 'Akito' roses during the 9 days of vase life. Treatments of thyme oil were stopped from day 3 to day 9. Only data for 0.78 mg/ml data is shown for day 6. Distilled water and 5% w/w glycerol treatments were stopped from day 6 and data is not shown for day 9. Data are means of three replications, ± SD.

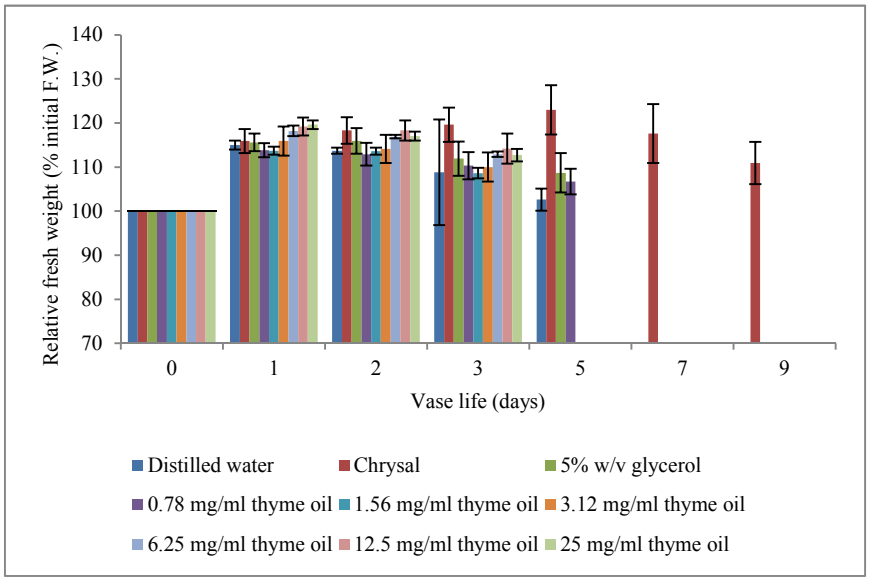


Figure 5.13: Changes in fresh weight of the 'Akito' roses during the 9 days of vase life. Treatments of thyme oil were stopped from day 3 to day 9.Only data for 0.78 mg/ml data is shown for day 6. Distilled water and 5% w/w glycerol treatments were stopped from day 6 and data is not shown for day 9. Data are means of three replications, ± SD.

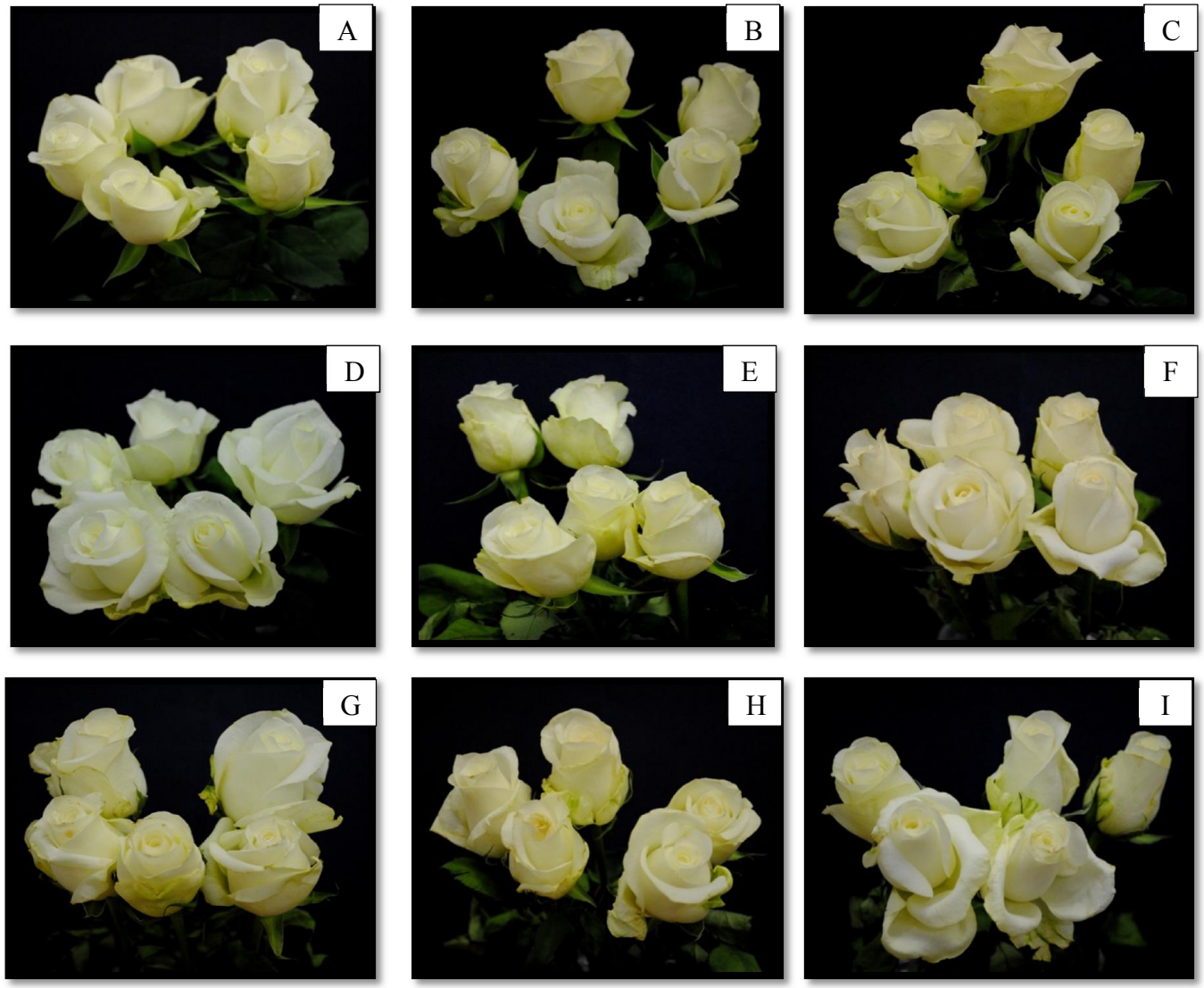


Figure 5.14: Overall appearances of 'Akito' roses held in distilled water(A), Chrysal (B), 5% glycerol (C), 0.78 mg/ mL thyme oil (D), 1.56 mg/ mL thyme oil (E), 3.12 mg/ mL thyme oil (F), 6.25 mg/ mL thyme oil (G), 12.5 mg/ mL thyme oil (H), and 25.0 mg/ mL thyme oil (I), at day 3. (Thyme oil treatments reach to an unaccepted stage before other treatments).

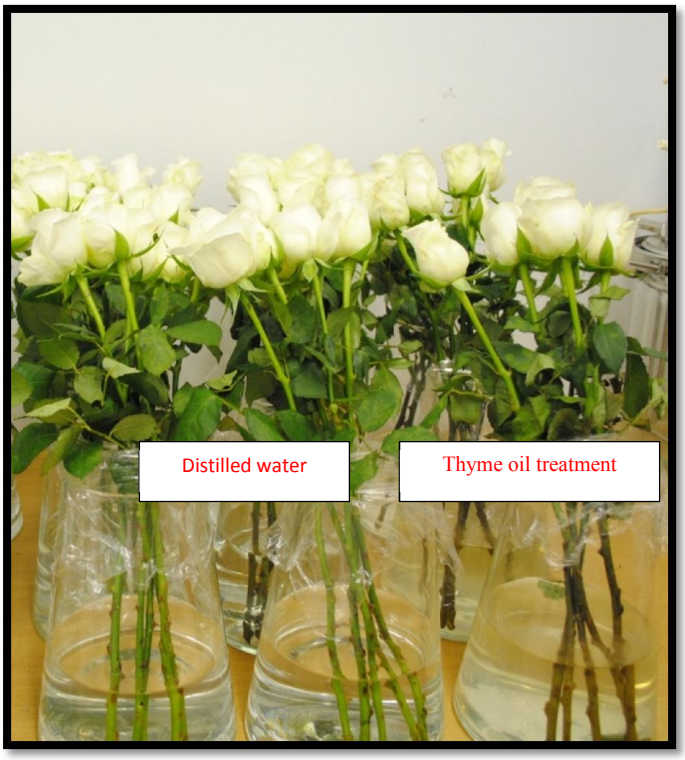


Figure 5.15: Blackening symptom on stems of 'Akito' roses held in thyme oil treatments.

5.3.2 Consideration of using weak organic acids combined with a low pH buffer on bacterial growth

The nine weak organic acids (Adipic acid, benzoic acid, Trans-cinnamic acid, ferulic acid, fumalic acid, gluconic acid lactone, propionic acid, sorbic acid and tartaric acid), some of which are used as preservatives in the food industry were studied for their effectiveness against bacterial growth. From the various bacteria (chapter 3) 6 out of 19 species were selected to be tested with weak organic acids.

The investigation of weak organic acids was divided into two experiments. In the first experiment, the weak organic acids were tested in an unbuffered system, while experiment 2 used a citric acid-phosphate buffer (pH4) to prepare the TSA.

5.3.2.1 Comparison of weak organic acids on bacterial growth in TSA pH 7.

To compare the effect of weak organic acids, six species of bacteria were inoculated on TSA. The final pH of the TSA was around 7 (unbuffered). Bacteria L4 (*Enterobacteria spp.*), L7 (*Staphylococcus spp.*), L8 (*Bacillus spp.*), L29 (*Pseudomonas spp*.), A1 (*Neisseria spp.)* and A36 (*Streptococcus spp.*) could all grow on this agar. (Table 5.2). The results showed that Trans-cinnamic acid alone could control the growth of some bacteria. However, Trans-cinnamic acid could control only L7, A1 and A36 at pH 7.

5.3.2.2 Comparison of weak organic acids on bacterial growth in TSA pH 4.

In this experiment, the TSA was buffered to pH 4 using a citric acid phosphate buffer. This was carried out because the performance of the weak acids is likely to be optimal under these conditions.

The results showed that only L8 (*Bacillus spp.*) could grow on the TSA at pH4, while, L4 (*Enterobacteria spp*.), L7 (*Staphylococcus spp.*), L29 (*Pseudomonas spp*.), A1 (*Neisseria spp.)* and A36 (*Streptococcus spp.*) could not grow at pH 4. A comparison of the effectiveness against bacterial growth of the nine weak organic acids showed that only Trans-cinnamic acid could control the growth of L8 (Table 5.3). This is in keeping with the previous experiment where trans-cinnamic was effective against some of the bacteria present. Interestingly, in combination with the buffer, trans-cinnamic acid was affective against L8, which had been resistant to it in unbuffered conditions. This suggests that trans-cinnamic acid, in combination with a buffer, may be a potential preservative for cut flowers.

Table 5.2: Zone of inhibition of bacteria when exposed to various weak organic acids at 1,000 ppm after 48 hours at 30°C on TSA (pH 7).

Microorganisms	**Mean zone of inhibition (mm)**									
	Distilled water	**Adipic acid**	**Benzoic acid**	**Trans-cinnamic acid**	**Ferulic acid**	**Fumalic acid**	**Gluconic acid lactone**	**Propionic acid**	**Sorbic acid**	**Tartaric acid**
Enterobacteria spp. (L4)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Staphylococcus spp. (L7)	0.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	0.0	0.0
Bacillus spp. (L8)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas spp. (L29)	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Neisseria spp. (A1)	0.0	0.0	0.0	3.0	0.0	0.0	0.0	0.0	0.0	0.0
Streptococcus spp. or Enterococcus spp. (A36)	0.0	0.0	0.0	2.0	0.0	0.0	0.0	0.0	0.0	0.0

Table 5.3: Zone of inhibition of bacteria when exposed to various weak organic acids at 1,000 ppm after 48 hours at 30°C on TSA (pH 4).

Microorganisms	**Mean zone of inhibition (mm)**									
	Distilled water	**Adipic acid**	**Benzoic acid**	**Trans-cinnamic acid**	**Ferulic acid**	**Fumalic acid**	**Gluconic acid lactone**	**Propionic acid**	**Sorbic acid**	**Tartaric acid**
Enterobacteria spp. (L4)*	-	-	-	-	-	-	-	-	-	-
Staphylococcus spp. (L7)*	-	-	-	-	-	-	-	-	-	-
Bacillus spp. (L8)	0.0	0.0	0.0	5.0	0.0	0.0	0.0	0.0	0.0	0.0
Pseudomonas spp. (L29)*	-	-	-	-	-	-	-	-	-	-
Neisseria spp. (A1)*	-	-	-	-	-	-	-	-	-	-
Streptococcus spp. or Enterococcus spp. (A36)*	-	-	-	-	-	-	-	-	-	-

Note: * L4, L7, L29, A1 and A36 could not grow on TSA (pH4)

5.4 Discussion

The use of antimicrobial compounds in the water is generally considered to extend the vase life of cut flowers such as roses, gerberas, gladioli and antirrhinums (Hoogerwerf and van Doorn, 1992). This study investigated new, alternative antibacterial compounds for the control of bacteria in vase water that are safe both for humans and the environment. Many plant species themselves produce essential oils that contain complex mixtures of secondary metabolites that play a role in chemical defence (Teixeira da Silva, 2003). Commercial essential oils from plants are often mixtures of several components; some of those components in oregano, clove, cinnamon, citral, garlic, coriander, rosemary, parsley, lemongrass, sage and vanillin have been shown to express antimicrobial effects (Tajkarimi *et al*., 2010).

This research project investigated the efficacy of twelve essential oils (West Indian bay, cinnamon leaf, clove bud, clove leaf, Chinese ginger, lemongrass, mandarin, rosemary, sage, spearmint, sweet fennel, and thyme). Various essential oils were screened for their effect on 18 bacterial species by comparing inhibition zones.

Thyme oil and lemongrass oil gave good effectiveness against a wide range of bacteria compared to other essential oils tested. Of these two, the minimal bactericide concentration (MBC) of thyme oil was lower than that of lemongrass oil. Therefore, thyme oil was selected for further study with cut flowers.

In the next study, the efficacy of thyme in the vase water of the 'Tiber' lily and 'Akito' rose was investigated, and found to be not effective. Thyme oil showed that its effectiveness against bacteria was very short lived, i.e. at day 0 only. Beyond day 0 it appeared to have a negative effect by supporting the growth of higher populations of bacteria. Moreover, thyme oil may even impart negative effects on the stems as they displayed blackening. It is unclear what the nature of the blackening was, but it may have been a necrotic effect caused directly by the presence of thyme oil, or perhaps a secondary effect of the increased numbers of bacteria present in the thyme oil treatments. Stem damage may cause additional chemical compounds to be released from these stems into the vase water (Zieslin, 1989). Bacterial growth may then be further increased after the leakage of carbohydrates (glucose, fructose,

sucrose) and proteins from the damaged plant tissue (Put, 1990). In addition, the pH of the thyme oil treatment was increased from 4 and was around 5 at the end of the vase life. The pH above 4 was suitable for bacterial growth (van Doorn, 1995). Although thyme oil gave an effectiveness in the agar plate study, it was not successful when applied to cut flowers. A limitation of this particular study may be the short time period selected for agar plate assay (standard protocol used). The time for the screening test and MBC was 48 h; however, bacteria may gain resistance grow up after two days. This is important because vase life studies extend for longer than two days, and the general expectancy is that flowers should have a vase life greater than seven days.

Kazemi *et al*. (2012) report that the application of 150 mg/ mL thyme oil in preservative solution for Lisianthus flowers could extend vase life to 14 days. However, this concentration of thyme oil is very high, and could not be used practically in a commercial situation. There is, however, scope for further work with thyme oil and perhaps other essential oils; they may have a positive effect when combined with other chemicals. The microbial populations in vase solutions of Lisianthus flowers treated with thyme oil (Thymus vulgaris) and 5-sulfosalicylic acid were lower than with other treatments (Kazemi and Ameri, 2012).

Organic acids and their esters; citric, tartaric, malic, lactic, acetic, propionic, sorbic, benzoic, para-hydroxybenzoate (parabens), are used as preservatives in the food industry (Garbutt, 1997). This research investigated the efficacy of weak organic acids in the preservation of cut flowers. The nine weak organic acids (adipic acid, benzoic acid, Trans-cinnamic acid, ferulic acid, fumalic acid, gluconic acid lactone, propionic acid, sorbic acid and tartaric acid) used as food preservatives were studied in vitro for their effect on bacterial growth. The six bacterial species were isolated from the vase water of ‘Tiber ’lilies and ‘Akito’ roses, and tested with various organic acids which were tested in buffered and unbuffered systems. Low pH is generally regarded to improve the vase life of cut flowers, and most flower foods contain an acid to reduce the pH of the vase solution (Zagory and Reid, 1986). In general it is believed that weak acids perform at their optimum level in buffered systems.

In the first experiment, bacteria were inoculated on to TSA that used distilled water for preparation. All bacteria could grow in this condition (pH 7). Generally,

bacteria grow better on a pH above 4 (van Doorn, 1995). Under this condition, Trans-cinnamic acid was the only one that acted effectively against bacteria. In the second experiment, bacterial samples were inoculated on TSA at pH 4 using a citric acid-phosphate buffer for preparation. At pH 4, only L8 (*Bacillus spp*.) could grow on this agar and Trans-cinnamic acid could operate against bacteria L8. This is an encouraging result since it indicates that this particular acid, in combination with a buffer system, was effective against all the bacteria tested. In fact, cinnamic acid is a natural organic acid that found in some fruit (i.e. cranberry and prune) and many spices (i.e. cinnamon and clove). Cinnamic acid had antimicrobial activity against spoilage microorganisms and pathogenic bacteria (Truong, 2007). The application of 10 mg/ L cinnamic acid in a solution of pH 3 has been shown to be effective at killing spoilage yeasts and mould (Anslow and Stratford, 2000).

5.5 Conclusions

The screening of 12 essential oils (West Indian bay, cinnamon leaf, clove bud, clove leaf, Chinese ginger, lemongrass, mandarin, rosemary, sage, spearmint, sweet fennel and thyme) showed that thyme oil and lemongrass oil are effective against more bacterial species over other essential oils. The comparison MBC of thyme oil and lemongrass oil showed that the average of MBC for thyme oil was lower than that of lemongrass. However, the thyme oil failed to extend the vase life of 'Tiber' lily and 'Akito' rose. Thyme oil was effective against bacteria for a very short time and could not extend vase life especially that of the 'Akito' rose.

Thyme oil may give impart effects on the stems as the stems showed blackening. It may also encourage the development of higher bacterial populations, perhaps by providing additional nutrients after the bacteria present have adapted to it.

The efficacy of nine weak organic acids was studied in vivo for their effect on six bacterial species. Bacterial samples were inoculated on TSA pH 4 and 7. At pH 7, the TSA was prepared by using distilled water. All six bacteria species grew well under these conditions. The results of weak organic acids studied at pH 7 showed that Trans-cinnamic acid was the only weak organic acid that could act against some bacteria. At pH 4, only L8 (*Bacillus spp*.) could grow under these conditions and

Trans-cinnamic acid was effective against this bacteria. The initial results of testing Trans-cinnamic acid against bacteria offered some promise for it to be applied to the vase water of cut flowers, in combination with buffer systems.

CHAPTER SIX

General discussion and conclusions

6.1 General discussion

This research project studied the way to extend the vase life of mixed flowers bouquets. Mixtures of lilies and roses were selected to be a case study for this project. The display life of mixed flowers bouquets depends on the individual life of each flower in the bouquet. The vase lives of flowers vary according to their genotype. The 'Tiber' lily was selected as an inflorescence-type of flower and the 'Akito' rose as a single-type of flower.

Previous research has studied various factors affecting vase life and the way to extend the vase life of only single species flowers. In fact, cut flowers are sold both in single species and mixed flowers in the market but there is less research focused on mixed flowers bouquets. However, when flower types are mixed, there may be factors that limit the vase life that are not present in single variety bouquets.

This project concentrates on the study of various factors influencing the vase life of mixed flowers bouquets. The mixture of 'Tiber' lilies and 'Akito' roses was investigated for its influence on quality and vase life. The results of this project have shown that not only does the phenotype of flowers have an influence on vase life but also that the accumulation of sugars in petals and leaves, and bacterial populations in vase water, all vary in relation to stages of bud opening, flower quality and vase life.

The first experiment observed changes in the overall appearance of nine cultivars of cut roses and two varieties of cut lilies throughout their vase life. For the cut roses, these changes were used to create a standard index for indicating bud opening, flower and leaf quality, while a flower quality index was created for the 'Oriental' lily. The indices were used in these studies to indicate the vase life of each cultivar in the individual experiments.

For cut roses, their vase lives varied among cultivars and this may be related to differences in genotype. Each cultivar had differences in appearance, colour and the

way they opened. Besides vase life, the contents of glucose, fructose, sucrose and myo-inositol were measured in the petals and leaves and were found to be different among cultivars. Sugars in petals and leaves were measured for their contents in relation to short-lived and long-lived cultivars. A correlation of all sugar contents in petals with vase lives shows that the level of sugar content may not relate to long-lived cultivars.

For cut lilies, individual bud life affected the vase life of the whole inflorescence. The two varieties of 'Oriental' lily studied (Mother's Choice and Tiber), showed differences in both vase life and the time to opening of an individual bud. However, 'Mother's Choice' and 'Tiber' had the same trend in terms of individual bud life. The primary and the secondary bud of both cultivars were not significantly different in their bud life. Therefore, individual bud life of the primary and secondary bud may be suitable to evaluate inflorescence longevity.

Moreover, the contents of glucose, fructose and sucrose were measured in the primary and the secondary bud of the 'Tiber' lily. Differences in the content of fructose glucose and sucrose between the primary and secondary bud did not relate to the longevity life of individual bud life. However, this experiment found increases in sucrose contents in both positions before the time of opening. This increase in sucrose may relate to the role of the carbohydrate energy source for bud opening.

The study into the role of sugars in the petals of both lily rose suggested that the level of all the sugar contents in petals were not related to long-lived cultivars of roses. However, the increase in sucrose level in individual buds of the lily may be related to the accumulation of an energy source. Therefore, sucrose may have an important role in flower development, such as bud opening, i.e. more than other sugars. Apart from this there were no strong correlations between levels of sugars and longevity of flowers. In fact, overall, the levels of sugars in tissues were surprisingly constant, showing little sign of fluxes between tissues (e.g. leaves to petals). It may be, therefore, that factors other than sugar levels influence the vase life characteristics of these cut flowers.

The second experiment investigated bacterial species and the influence of their population on the vase life of single and mixed flowers bouquets. Differences in

numbers of stem of ‘Akito’ roses, ‘Valentino’ roses, ‘Tiber’ lilies and a mixture of ‘Tiber’ lilies with ‘Akito’ roses were investigated. The results showed that a number of species from a restricted number of groups were found in the vase water of ‘Akito’ roses, ‘Valentino’ roses and ‘Tiber’ lilies but the mixed species varied, dependent on the flower varieties present, and were also different again in mixtures. This may reflect different conditions which are maintained by different flower types, and their combinations. Most interestingly, mixtures of flower varieties resulted in a more restricted (less diverse) bacterial population.

Bacterial populations in vase water increased when there were more stems in the vase, but only for mixed stem bouquets. Where there were more bacterial populations in vase water this could decrease the longevity of flowers. As expected, bacteria population was reduced when flowers were held in commercial liquid flower food.

Observation of bacteria on the cut surface, outer stem and in the xylem indicated that at 5 cm the base of the stem had greater numbers of bacteria than in other parts. This experiment clearly showed that bacteria were able to actively enter the xylem vessels and travel up the stem, which could lead to changes in a number of processes in the plants. Further, active colonization of the stem surfaces below the water level was demonstrated in this study. Van Doorn and de Witte (1991) also found that bacterial occlusion was located in the basal 5 cm of the stem. This was associated with a decrease in hydraulic conductance and the limitation of water uptake in cut rose stems.

Thus placing a bouquet with more stems in vase water may mean there are more bacteria in that water and that the end of vase life will be accelerated. Preventing xylem occlusion and controlling bacterial growth in vase water is, therefore, a good practice for extending vase life.

The influence of chemical exudates was studied in experiment three. Placing ‘Tiber’ lilies and ‘Akito’ roses together may have an influence on the quality and vase life of each of them. This experiment separated the testing of chemical exudates into two groups. The first group, ‘Tiber’ lilies were held in the old water that had been

collected from ‘Akito’ roses. The second group was ‘Akito’ roses that were held in the old water from ‘Tiber’ lilies.

The results showed that the role of chemical exudates on flowers was not clear in the present study as they only had an effect during the early part of the vase life. The vase lives of ‘Tiber’ lilies and ‘Akito’ roses in all treatments were not significantly different because the bacterial population increased later after flowers were held in the vase. The appearance of these bacteria was in spite of the use of sterilized vase water (filter sterilized to prevent damage to chemical constituents in the water) and the surface sterilization of the plant stems. The increase of bacteria may have been the most important factor that limited the vase life of flowers, which probably masked any effects from the chemical exudates. The contamination by bacteria may come from bacteria that accumulate in the xylem (van Doorn, 1995). Such bacteria would be difficult to remove with the chemical treatment which was used on stems in this study. In fact, it is clearly difficult to maintain bacteria free (aseptic) conditions in experiments such as this, and future studies in this area could usefully work towards this end.

The last experiment studied the efficacy of essential oils and weak organic acids as an antimicrobial. The results showed that thyme oil was effective against bacteria only in the agar plate study. However, its efficacy did not work when applied to vase water. Thyme oil could not reduce the increase in bacteria and extend vase life. The efficacy of thyme oil was very short and could not control the bacterial population throughout vase life. A relatively low concentration of thyme oil was tested in these studies because it was important to work at a concentration that could be commercially viable.

In fact, thyme oil appeared to have a detrimental effect by both increasing the number of bacteria present and causing stem blackening. The thyme oil treatments may have given a very direct demonstration of the role of bacterial populations on vase life because the higher concentrations both increased bacterial population numbers and significantly reduced vase life (although the influence on vase life may have been due to effects other than bacterial numbers).

Thyme oil was effective against bacteria for a very short time. Therefore, thyme oil may be effective when applied for cleaning purposes. Solorzano-Santos and Miranda-Novales (2011) suggest that using essential oils as part of washing solutions could reduce number of bacteria, which decreased the risk of pathogen contamination from fruit or vegetables to the washing solutions. Perhaps it could be used as part of a pre-treatment of vases etc., which would then have the added benefit of providing a 'room fragrance'.

For weak organic acids, Trans-cinnamic acid was more effective against bacteria than other acids. Trans-cinnamic acid may be effective against bacteria when included with vase water, in combination with pH buffering. The control of bacteria in vase water by adjusting the pH down to 3 to 4 is a common practice with cut flowers. Using a low buffer and/or organic acid, such as citric acid and Trans-cinnamic acid, may be effective against bacteria and is also practical for cut flower practice. In general this project has indicated the importance of bacterial populations in vase life, and so future research in this area should work towards systems that are able to eliminate bacteria from vase water whilst using safe, environmentally friendly chemicals, such as essential oils and weak organic acids.

6.2 General conclusions

The consideration of various factors that influence the vase life of mixed flowers bouquets was studied by mixing 'Tiber' lilies and 'Akito' roses. The results indicated that the differences in vase life of each flower in mixed bouquets may come from the influence of their genotype. Besides the acumulatin of sucrose in lily buds, the sugars contents studied in this experiment were not related to long-lived cultivars.

Bacteria accumulating in vase water (and perhaps also in the xylem) appear to be the main causes of the short life of flowers. Besides bacteria, chemicals exuding from the stems of flowers (particularly in mixtures) may have an effect on other flowers in the same vase. However, the role of chemical exudates on flowers was not clear in the present study as they only had an effect during the early part of the vase life, before the developing bacterial population began to have an effect.

Using essential oils, such as thyme oil, for the control of bacteria was not effective when applied in real work with cut flowers.

Trans-cinnamic acid may, however, have a good effect when added to vase water in the presence of a buffer system.

6.3 Recommendations for future work

- The relation between sugar content and long-lived cultivars in this project was not clear. The data should be collected from more crops and should be collected every season. This may give greater detail in explaining the role of sugars on the longevity of flowers.
- Studies into the influence of chemical exudates on vase life and quality of flowers should eliminate the influence of bacteria in vase water. Such studies may give clearer data for explaining the influence of chemical exudates on vase life. Moreover, chemical compounds are exuded into the water should be investigated. This may give more information for this study.
- The control of bacteria in vase water by using antimicrobial compounds in combination with essential oils (or combinations of essential oils) may be more effective against bacteria than using essential oils alone.
- Buffered Trans-cinnamic acid should be observed in cut 'Tiber' lilies and 'Akito' roses.

6.4 Application of this research project

- Due to size of bacterial population was increased by number of stems, changing vase water every 2-3 days may help to reduce accumulation of bacteria in vase water. Moreover, recut 5 cm above the cut end could remove bacteria in xylems.
- For the company, cleaning stems of flower with antimicrobial compounds could eliminate some of bacteria that accumulate at suface of stems. This may extend longevity of cut flowers.

CHAPTER SEVEN

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APPENDICES

APPENDIX A

Identification of bacterial species

A.1. Identification of methods

Bacterial samples isolated from the vase water described in chapter three were identified by using media classification (MacConkey, Pseudomonas selective age and Columbia CNA), gram's stain and biochemical test. A flowchart example of a bacteria identification scheme is shown in picture A.1. Moreover, Colony morphology of some organisms grown on selective media is shown in picture A.2.

A.1.1. Gram's stain

Samples of bacteria from cultures on solid media with a loop are smeared on a small drop of water. Leave the slide to dry in air, then heat-fix by passing the slide quickly through flame three times.

Gram microbiology staining kit (Fisher Scientific) was used for gram's stain. Stain with methyl violet solution for 20 s. Wash off and replace with iodine solution. Leave for 1 min. Wash off iodine solution with 95% alcohol, leaving on for a few seconds only. Wash with water. Stain with the safranin solution for 2 min (Harrigan, 1998).

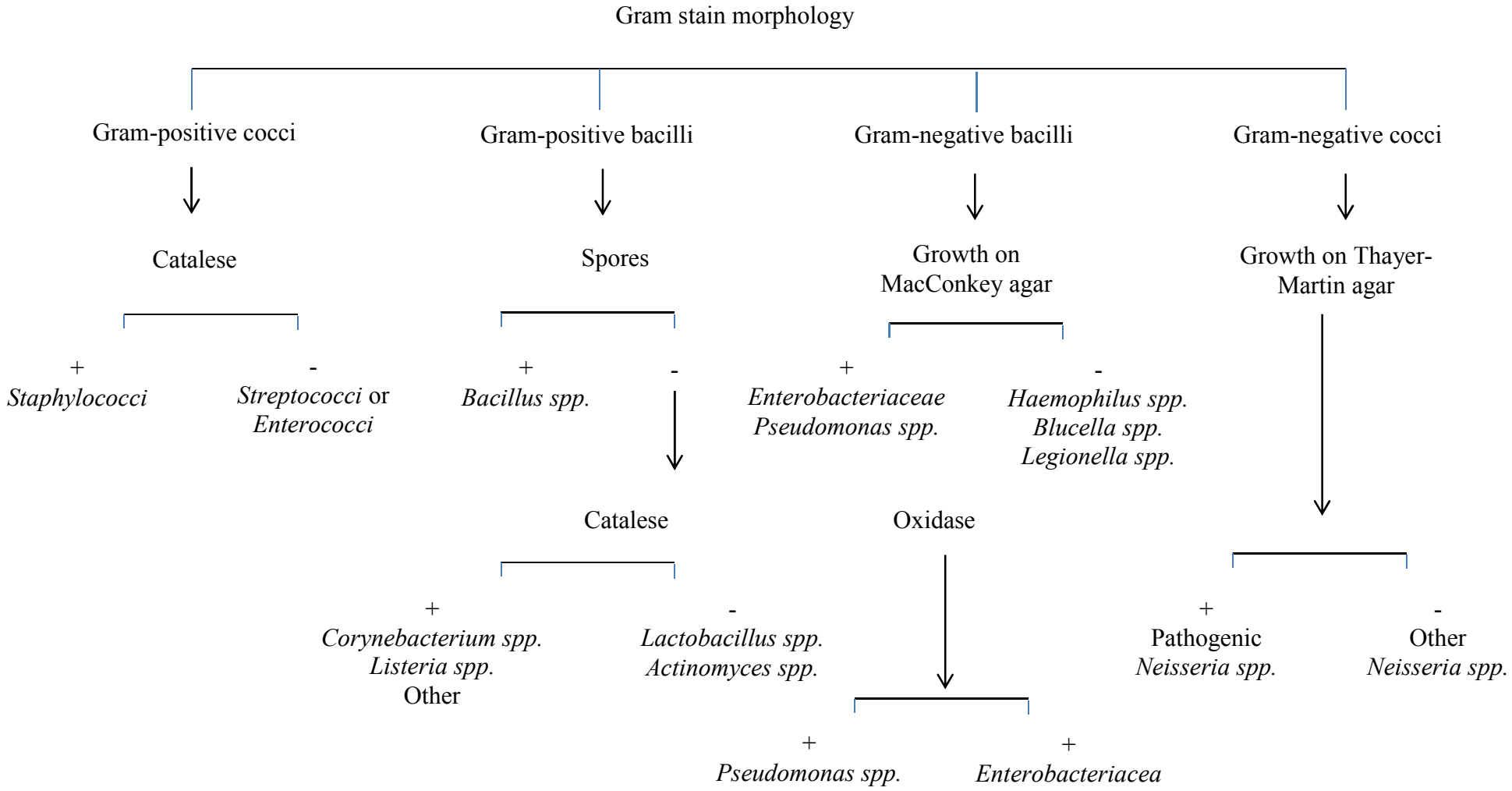
A.1.1.2 biochemical tests

(a) Oxidase test

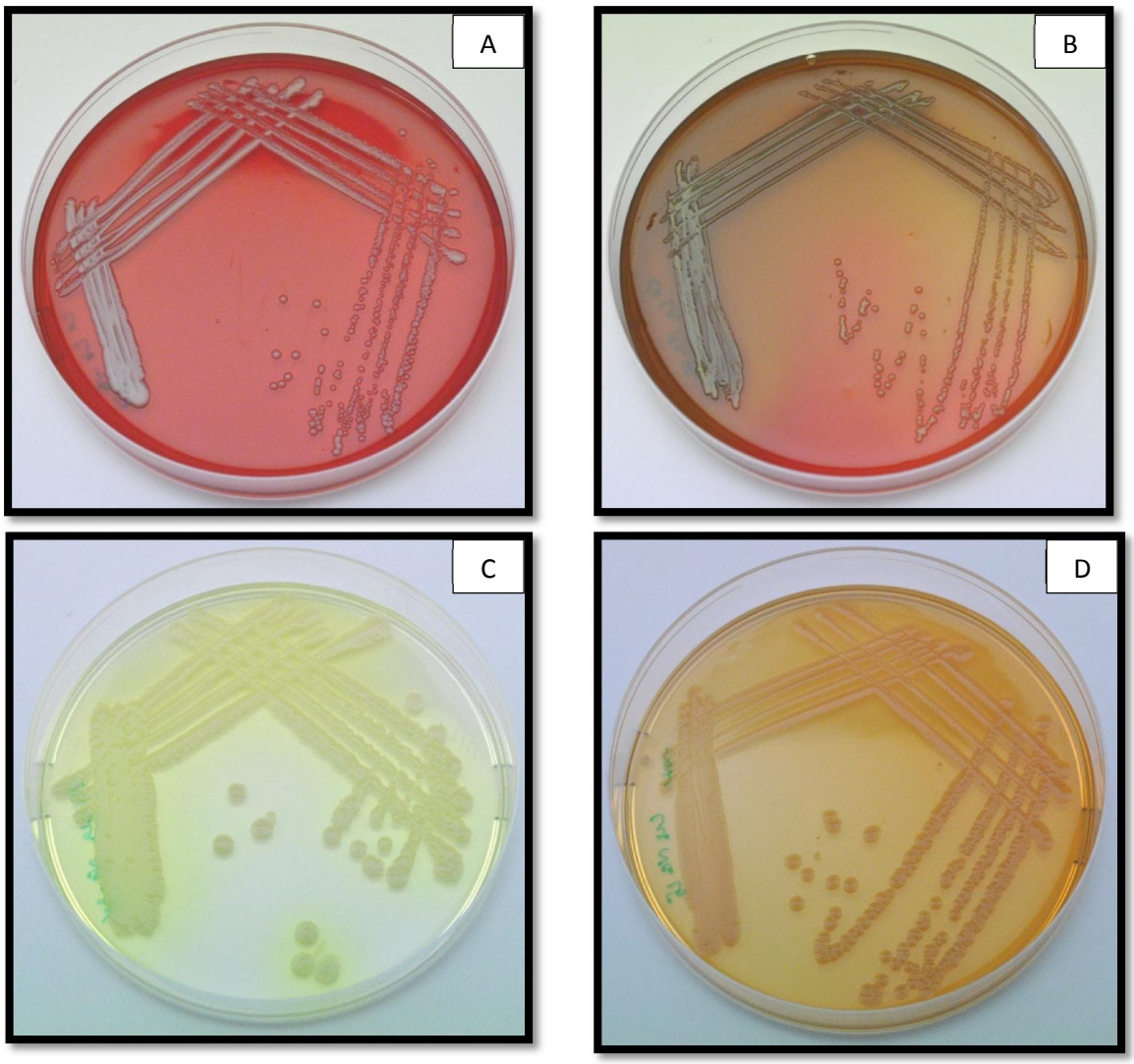
Take a loop of bacterial sample, and then spread onto an oxidase disc (oxidase test disc, 70439 Oxidase Test, Fluka). The reaction is observed for 2 min at 25-30°C. The positive expresses a deep purple blue while no colour for negative.

(b) Catalase test

Put a loop of bacterial sample onto a glass slide and then add a few drops of 3% hydrogen peroxide solution (Fluka). A positive test shows a quick appearance of bubbling (Lammert, 2007).



Picture A.1: Flowchart example of a bacteria identification scheme (Source: Forbes *et al*., 2007).



A.2: Colony morphology of some organisms grown on selective media; *Staphylococcus spp*. (L8) on Columbia CNA (A); *Streptococcus spp.* or *Enterococcus spp.* (L9) on Columbia CNA (B); *Pseudomonas spp.* (L29) on *Pseudomonas* selective agar (C); *Enterobacteria spp.* (L4) on MacConkey (D).

APPENDIX B

Statistical tables

ANOVA tables for chapter 2

Experiment 1: Temporal changes in sugar content and overall appearance during vase life of nine cultivars of cut rose.

Table B.1.1: ANOVA table for vase life of nine cultivars of cut roses.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	240.148	8	30.019	16.992	0.000
Error	79.500	45	1.767		
Total	3509.000	54			

Table B.1.2: ANOVA table for changes in fructose in the petals of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	19087.949	8	2385.994	25.426	0.000
Error	1689.139	18	93.841		
Total	201961.190	27			

Table B.1.3: ANOVA table for changes in fructose in the petals of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	9770.782	8	1221.348	12.389	0.000
Error	1774.450	18	98.581		
Total	186908.266	27			

Table B.1.4: ANOVA table for changes in fructose in the petals of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	14540.360	8	1817.545	37.653	0.000
Error	868.883	18	48.271		
Total	209557.147	27			

Table B.1.5: ANOVA table for changes in fructose in the petals of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	10137.505	8	1267.188	18.652	0.000
Error	1222.920	18	67.940		
Total	143673.026	27			

Table B.1.6: ANOVA table for changes in fructose in the petals of nine cultivars of cut roses on day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	5762.645	8	720.331	1.691	0.169
Error	7666.262	18	425.903		
Total	152927.861	27			

Table B.1.7: ANOVA table for changes in glucose in the petals of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	9437.331	8	1179.666	22.910	0.000
Error	926.827	18	51.490		
Total	74561.574	27			

Table B.1.8: ANOVA table for changes in glucose in the petals of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	6597.305	8	824.663	8.729	0.000
Error	1700.559	18	94.475		
Total	66835.890	27			

Table B.1.9: ANOVA table for changes of changes in glucose in the petals of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	6823.246	8	852.906	40.169	0.000
Error	382.194	18	21.233		
Total	50620.908	27			

Table B.1.10: ANOVA table for changes in glucose in the petals of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	3158.799	8	394.850	17.517	0.000
Error	405.736	18	22.541		
Total	30031.818	27			

Table B.1.11: ANOVA table for changes in glucose in the petals of nine cultivars of cut roses on day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	2134.734	8	266.842	14.060	0.000
Error	341.606	18	18.978		
Total	23782.325	27			

Table B.1.12: ANOVA table for changes in sucrose in the petals of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	602.900	8	75.363	41.425	0.000
Error	32.747	18	1.819		
Total	1667.831	27			

Table B.1.13: ANOVA table for changes in sucrose in the petals of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	326.383	8	40.798	15.913	0.000
Error	46.148	18	2.564		
Total	944.404	27			

Table B.1.14: ANOVA table for changes in sucrose in the petals of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	564.725	8	70.591	30.987	0.000
Error	41.006	18	2.278		
Total	2810.045	27			

Table B.1.15: ANOVA table for changes in sucrose in the petals of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	227.320	8	28.415	38.266	0.000
Error	13.366	18	0.743		
Total	1712.996	27			

Table B.1.16: ANOVA table for changes in sucrose in the petals of nine cultivars of cut roses on day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	168.383	8	21.048	12.432	0.000
Error	30.474	18	1.693		
Total	782.757	27			

Table B.1.17: ANOVA table for changes in myo-inositol in the petals of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	307.792	8	38.474	36.935	0.000
Error	18.750	18	1.042		
Total	1322.447	27			

Table B.1.18: ANOVA table for changes in myo-inositol in the petals of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	106.632	8	13.329	6.524	0.000
Error	36.777	18	2.043		
Total	1251.762	27			

Table B.1.19: ANOVA table for changes in myo-inositol in the petals of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	310.558	8	38.820	20.127	0.000
Error	34.718	18	1.929		
Total	2310.502	27			

Table B.1.20: ANOVA table for changes in myo-inositol in the petals of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	51.576	8	6.447	7.990	0.000
Error	14.525	18	0.807		
Total	375.913	27			

Table B.1.21: ANOVA table for changes in myo-inositol in the petals of nine cultivars of cut roses on day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	69.928	8	8.741	17.311	0.000
Error	9.089	18	0.505		
Total	351.543	27			

Table b.1.22: ANOVA table for changes in fructose in the foliage of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	1634.854	8	204.357	24.964	0.000
Error	147.350	18	8.186		
Total	2261.428	27			

Table B.1.23: ANOVA table for changes in fructose in the foliage of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	338.263	8	42.283	4.787	0.003
Error	158.978	18	8.832		
Total	848.747	27			

Table B.1.24: ANOVA table for changes in fructose in the foliage of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	367.046	8	45.881	7.600	0.000
Error	108.662	18	6.037		
Total	1238.065	27			

Table B.1.25: ANOVA table for changes in fructose in the foliage of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	918.168	8	114.771	4.854	0.003
Error	425.563	18	23.642		
Total	4078.040	27			

Table B.1.26: ANOVA table for changes of changes in glucose in the foliage of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	1383.357	8	172.920	11.148	.000
Error	279.212	18	15.512		
Total	2752.675	27			

Table B.1.27: ANOVA table for changes of changes in glucose in the foliage of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	920.706	8	115.088	113.351	.000
Error	18.276	18	1.015		
Total	3115.554	27			

Table B.1.28: ANOVA table for changes of changes in glucose in the foliage of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	1598.312	8	199.789	48.295	.000
Error	74.462	18	4.137		
Total	4359.790	27			

Table B.1.29: ANOVA table for changes of changes in glucose in the foliage of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	343.942	8	42.993	1.800	0.143
Error	430.035	18	23.891		
Total	2156.001	27			

Table B.1.30: ANOVA table for changes of changes in sucrose in the foliage of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	8772.804	8	1096.600	20.304	0.000
Error	972.160	18	54.009		
Total	31327.130	27			

Table B.1.31: ANOVA table for changes of changes in sucrose in the foliage of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	3496.963	8	437.120	6.976	0.000
Error	1127.819	18	62.657		
Total	14370.642	27			

Table B.1.32: ANOVA table for changes of changes in sucrose in the foliage of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	3177.448	8	397.181	12.997	0.000
Error	550.081	18	30.560		
Total	11174.290	27			

Table B.1.33: ANOVA table for changes of changes in sucrose in the foliage of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	3382.018	8	422.752	3.865	0.008
Error	1968.675	18	109.371		
Total	18187.405	27			

Table B.1.34: ANOVA table for changes of changes in sucrose in the foliage of nine cultivars of cut roses on day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	1817.236	8	227.154	2.736	0.036
Error	1494.624	18	83.035		
Total	33488.118	27			

Table B.1.35: ANOVA table for changes of changes in myo-inositol in the foliage of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	141.884	8	17.735	4.396	0.004
Error	72.628	18	4.035		
Total	13054.278	27			

Table B.1.36: ANOVA table for changes of changes in myo-inositol in the foliage of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	17.748	8	2.218	0.881	0.550
Error	45.311	18	2.517		
Total	8786.236	27			

Table B.1.37: ANOVA table for changes of changes in myo-inositol in the foliage of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	37.186	8	4.648	1.969	0.111
Error	42.495	18	2.361		
Total	7621.405	27			

Table B.1.38: ANOVA table for changes of changes in myo-inositol in the foliage of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	46.625	8	5.828	2.788	0.034
Error	37.623	18	2.090		
Total	4385.397	27			

Table B.1.39: ANOVA table for changes of changes in myo-inositol in the foliage of nine cultivars of cut roses on day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	63.242	8	7.905	2.911	0.028
Error	48.874	18	2.715		
Total	3907.553	27			

Table B.1.40: ANOVA table for changes of total sugars content in the petals of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	57385.273	8	7173.159	23.497	0.000
Error	5495.027	18	305.279		
Total	614488.620	27			

Table B.1.41: ANOVA table for changes of total sugars content in the petals of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	27878.292	8	3484.786	7.639	0.000
Error	8211.553	18	456.197		
Total	551465.330	27			

Table B.1.42: ANOVA table for changes of total sugars content in the petals of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	31705.020	8	3963.127	24.710	0.000
Error	2886.940	18	160.386		
Total	582604.240	27			

Table B.1.43: ANOVA table for changes of total sugars content in the petals of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	20768.533	8	2596.067	14.507	0.000
Error	3221.073	18	178.949		
Total	363215.420	27			

Table B.1.44: ANOVA table for changes of total sugars content in the petals of nine cultivars of cut roses on day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	12580.836	8	1572.605	2.778	0.034
Error	10191.053	18	566.170		
Total	336513.010	27			

Table B.1.45: ANOVA table for changes of total sugars content in the foliage of nine cultivars of cut roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	24453.934	8	3056.742	38.347	0.000
Error	1434.833	18	79.713		
Total	126526.640	27			

Table B.1.46: ANOVA table for changes of total sugars content in the foliage of nine cultivars of cut roses on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	6850.296	8	856.287	8.694	0.000
Error	1772.940	18	98.497		
Total	75504.660	27			

Table B.1.47: ANOVA table for changes of total sugars content in the foliage of nine cultivars of cut roses on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	7775.236	8	971.905	13.934	0.000
Error	1255.500	18	69.750		
Total	71835.360	27			

Table B.1.48: ANOVA table for changes of total sugars content in the foliage of nine cultivars of cut roses on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	9955.439	8	1244.430	5.999	0.001
Error	3733.993	18	207.444		
Total	85506.330	27			

Table B.1.49: ANOVA table for changes of total sugars content in the foliage of nine cultivars of cut roses on day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Variety	2424.754	8	303.094	3.597	0.011
Error	1516.820	18	84.268		
Total	59311.770	27			

Experiment II: Overall appearance changes during vase life of two cultivars of cut lily and changes in sugar content at different positions.

Table B.1.50: ANOVA table for change of fructose in sepals of 'Tiber' lily on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
position	1466.094	1	1466.094	19.005	0.012
Error	308.566	4	77.142		
Total	47155.606	6			

Table B.1.51: ANOVA table for change of fructose in sepals of 'Tiber' lily on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
position	757.127	1	757.127	4.228	0.109
Error	716.255	4	179.064		
Total	26082.673	6			

Table B.1.52: ANOVA table for change of fructose in sepals of 'Tiber' lily on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
position	1803.360	1	1803.360	39.920	0.003
Error	180.697	4	45.174		
Total	35273.638	6			

Table B.1.53: ANOVA table for change of fructose in sepals of 'Tiber' lily on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
position	1593.814	1	1593.814	47.072	0.002
Error	135.437	4	33.859		
Total	29268.271	6			

Table B.1.54: ANOVA table for change of fructose in sepals of 'Tiber' lily on day 8.

Source of variation	s.s.	df	m.s.	F	Sig.
position	96.962	1	96.962	3.644	0.129
Error	106.449	4	26.612		
Total	18364.413	6			

Table B.1.55: ANOVA table for change of glucose in sepals of 'Tiber' lily on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
position	1142.640	1	1142.640	14.386	0.019
Error	317.711	4	79.428		
Total	32105.257	6			

Table B.1.56: ANOVA table for change of glucose in sepals of 'Tiber' lily on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
position	398.046	1	398.046	4.298	0.107
Error	370.415	4	92.604		
Total	12089.794	6			

Table B.1.57: ANOVA table for change of glucose in sepals of 'Tiber' lily on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
position	1034.381	1	1034.381	17.342	0.014
Error	238.588	4	59.647		
Total	16598.719	6			

Table B.1.58: ANOVA table for change of glucose in sepals of 'Tiber' lily on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
position	674.160	1	674.160	64.814	0.001
Error	41.606	4	10.401		
Total	12354.657	6			

Table B.1.59: ANOVA table for change of glucose in sepals of 'Tiber' lily on day 8.

Source of variation	s.s.	df	m.s.	F	Sig.
position	272.296	1	272.296	24.326	0.008
Error	44.774	4	11.193		
Total	6810.214	6			

Table B.1.60: ANOVA table for change of sucrose in sepals of 'Tiber' lily on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
position	1.750	1	1.750	0.452	0.538
Error	15.491	4	3.873		
Total	1837.282	6			

Table B.1.61: ANOVA table for change of sucrose in sepals of 'Tiber' lily on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
position	4.717	1	4.717	1.619	0.272
Error	11.652	4	2.913		
Total	850.865	6			

Table B.1.62: ANOVA table for change of sucrose in sepals of 'Tiber' lily on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
position	25.709	1	25.709	11.176	0.029
Error	9.201	4	2.300		
Total	842.735	6			

Table B.1.63: ANOVA table for change of sucrose in sepals of 'Tiber' lily on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
position	190.181	1	190.181	50.375	0.002
Error	15.101	4	3.775		
Total	1417.964	6			

Table B.1.64: ANOVA table for change of sucrose in sepals of 'Tiber' lily on day 8.

Source of variation	s.s.	df	m.s.	F	Sig.
position	107.950	1	107.950	4.073	0.114
Error	106.018	4	26.505		
Total	7987.809	6			

Table B.1.65: ANOVA table for change of total sugars in sepals of 'Tiber' lily on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
position	5389.805	1	5389.805	14.871	0.018
Error	1449.756	4	362.439		
Total	192375.379	6			

Table B.1.66: ANOVA table for change of total sugars in sepals of 'Tiber' lily on day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
position	2052.390	1	2052.390	3.613	0.130
Error	2272.133	4	568.033		
Total	89683.677	6			

Table B.1.67: ANOVA table for change of total sugars in sepals of 'Tiber' lily on day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
position	6353.760	1	6353.760	27.720	0.006
Error	916.841	4	229.210		
Total	119274.410	6			

Table B.1.68: ANOVA table for change of total sugars in sepals of 'Tiber' lily on day 4.

Source of variation	s.s.	df	m.s.	F	Sig.
position	6348.555	1	6348.555	60.936	0.001
Error	416.739	4	104.185		
Total	102038.934	6			

Table B.1.69: ANOVA table for change of total sugars in sepals of 'Tiber' lily on day 8.

Source of variation	s.s.	df	m.s.	F	Sig.
position	254.541	1	254.541	2.451	0.192
Error	415.374	4	103.844		
Total	92787.088	6			

B.2 ANOVA tables for chapter 3

Experiment I: Bacteria population in vase water and the vase life of the cut 'Tiber' lily.

Table B.2.1: ANOVA table for changes of total bacteria plate count of cut 'Tiber' lilies on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.602	1	1.602	9.330	0.038
Error	0.687	4	0.172		
Total	16.090	6			

Table B.2.2: ANOVA table for changes of total bacteria plate count of cut 'Tiber' lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.135	1	0.135	0.403	0.560
Error	1.340	4	.335		
Total	37.490	6			

Table B.2.3: ANOVA table for changes of total bacteria plate count of cut 'Tiber' lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.082	1	0.082	4.900	0.091
Error	0.067	4	0.017		
Total	43.350	6			

Table B.2.4: ANOVA table for changes of total bacteria plate count of cut 'Tiber' lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.015	1	0.015	0.050	0.834
Error	1.193	4	0.298		
Total	35.290	6			

Table B.2.5: ANOVA table for changes of total bacteria plate count of cut 'Tiber' lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.375	1	0.375	5.769	0.074
Error	0.260	4	0.065		
Total	72.050	6			

Table B.2.6: ANOVA table for changes of *Staphylococcus spp*. (L7) in vase water of cut lilies on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	-	-
Error	0.000	4	0.000		
Total	24.000	6			

Table B.2.6: ANOVA table for changes of *Staphylococcus spp*. (L7) in vase water of cut lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.027	1	0.027	0.471	0.530
Error	0.227	4	0.057		
Total	42.920	6			

Table B.2.6: ANOVA table for changes of *Staphylococcus spp*. (L7) in vase water of cut lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.015	1	0.015	0.257	0.639
Error	0.233	4	0.058		
Total	41.330	6			

Table B.2.6: ANOVA table for changes of *Staphylococcus spp*. (L7) in vase water of cut lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.107	1	0.107	2.207	0.212
Error	0.193	4	0.048		
Total	32.040	6			

Table B.2.6: ANOVA table for changes of *Staphylococcus spp*. (L7) in vase water of cut lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.215	1	1.215	2.430	0.194
Error	2.000	4	0.500		
Total	42.230	6			

Table B.2.7: ANOVA table for changes of *Staphylococcus spp*. (L8) in vase water of cut lilies on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.015	1	0.015	0.529	0.507
Error	0.113	4	0.028		
Total	51.170	6			

Table B.2.8: ANOVA table for changes of *Staphylococcus spp*. (L8) in vase water of cut lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.735	1	0.735	49.000	0.002
Error	0.060	4	0.015		
Total	33.930	6			

Table B.2.9: ANOVA table for changes of *Staphylococcus spp*.. (L8) in vase water of cut lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.135	1	0.135	0.802	0.421
Error	0.673	4	0.168		
Total	37.810	6			

Table B.2.10: ANOVA table for changes of *Staphylococcus spp*. (L8) in vase water of cut lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.135	1	0.135	-	-
Error	0.000	4	0.000		
Total	27.870	6			

Table B.2.11: ANOVA table for changes of *Staphylococcus spp*. (L8) in vase water of cut lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.240	1	0.240	2.286	0.205
Error	0.420	4	0.105		
Total	29.700	6			

Table B.2.12: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (L9) in the vase water of cut lilies on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	0.000	1.000
Error	0.080	4	0.020		
Total	54.080	6			

Table B.2.13: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (L9) in the vase water of cut lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.807	1	0.807	2.396	0.197
Error	1.347	4	0.337		
Total	38.660	6			

Table B.2.14: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (L9) in the vase water of cut lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.375	1	0.375	-	-
Error	0.000	4	0.000		
Total	63.750	6			

Table B.2.15: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (L9) in the vase water of cut lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.327	1	0.327	3.769	0.124
Error	0.347	4	0.087		
Total	39.180	6			

Table B.2.16: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (L9) in the vase water of cut lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.482	1	0.482	5.898	0.072
Error	0.327	4	0.082		
Total	35.850	6			

Table B.2.17: ANOVA table for changes of *Enterobacteria spp.* (L4) in the vase water of cut 'Tiber' lilies on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.107	1	0.107	5.818	0.073
Error	0.073	4	0.018		
Total	26.640	6			

Table B.2.18: ANOVA table for changes of *Enterobacteria spp.* (L4) in the vase water of cut 'Tiber' lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.282	1	0.282	42.250	0.003
Error	0.027	4	0.007		
Total	29.790	6			

Table B.2.19: ANOVA table for changes of *Enterobacteria spp.* (L4) in the vase water of cut 'Tiber' lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.082	1	0.082	2.579	0.184
Error	0.127	4	0.032		
Total	68.890	6			

Table B.2.20: ANOVA table for changes of *Enterobacteria spp.* (L4) in the vase water of cut 'Tiber' lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	0.000	
Error	0.713	4	0.178		1.000
Total	64.740	6			

Table B.2.21: ANOVA table for changes of *Enterobacteria spp.* (L4) in the vase water of cut 'Tiber' lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.060	1	0.060	0.679	0.456
Error	0.353	4	0.088		
Total	63.140	6			

Table B.2.22: ANOVA table for changes of *Pseudomonas spp.* (L15) in vase water of cut 'Tiber' lilies on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.135	1	0.135	0.779	0.427
Error	0.693	4	0.173		
Total	149.830	6			

Table B.2.23: ANOVA table for changes of *Pseudomonas spp.* (L15) in vase water of cut 'Tiber' lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.960	1	0.960	6.472	0.064
Error	0.593	4	0.148		
Total	167.980	6			

Table B.2.24: ANOVA table for changes of *Pseudomonas spp.* (L15) in vase water of cut 'Tiber' lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.002	1	0.002	0.020	0.894
Error	0.333	4	0.083		
Total	130.070	6			

Table B.2.25: ANOVA table for changes of *Pseudomonas spp.* (L15) in vase water of cut 'Tiber' lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.107	1	0.107	2.286	0.205
Error	0.187	4	0.047		
Total	140.460	6			

Table B.2.26: ANOVA table for changes of *Pseudomonas spp.* (L15) in vase water of cut 'Tiber' lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.135	1	0.135	2.531	0.187
Error	0.213	4	0.053		
Total	139.550	6			

Table B.2.27: ANOVA table for changes of *Pseudomonas spp.* (L29) in vase water of cut 'Tiber' lilies on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.602	1	1.602	9.330	0.038
Error	0.687	4	0.172		
Total	16.090	6			

Table B.2.28: ANOVA table for changes of *Pseudomonas spp.* (L29) in vase water of cut 'Tiber' lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.135	1	0.135	0.403	0.560
Error	1.340	4	0.335		
Total	37.490	6			

Table B.2.29: ANOVA table for changes of *Pseudomonas spp.* (L29) in vase water of cut 'Tiber' lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.082	1	0.082	4.900	0.091
Error	0.067	4	0.017		
Total	43.350	6			

Table B.2.30: ANOVA table for changes of *Pseudomonas spp.* (L29) in vase water of cut 'Tiber' lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.015	1	0.015	0.050	0.834
Error	1.193	4	0.298		
Total	35.290	6			

Table B.2.31: ANOVA table for changes of *Pseudomonas spp.* (L29) in vase water of cut 'Tiber' lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.375	1	0.375	5.769	0.074
Error	0.260	4	0.065		
Total	72.050	6			

Table B.2.32: ANOVA table for changes of *Brucella spp*. (L10) in the vase water of cut 'Tiber' lilies on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.082	1	0.082	1.043	0.365
Error	0.313	4	0.078		
Total	30.770	6			

Table B.2.33: ANOVA table for changes of *Brucella spp*. (L10) in the vase water of cut 'Tiber' lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.540	1	0.540	15.429	0.017
Error	0.140	4	0.035		
Total	32.420	6			

Table B.2.34: ANOVA table for changes of *Brucella spp*. (L10) in the vase water of cut 'Tiber' lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2.282	1	2.282	21.062	0.010
Error	0.433	4	0.108		
Total	48.090	6			

Table B.2.35: ANOVA table for changes of *Brucella spp*. (L10) in the vase water of cut 'Tiber' lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.707	1	1.707	33.032	0.005
Error	0.207	4	0.052		
Total	46.740	6			

Table B.2.36: ANOVA table for changes of *Brucella spp*. (L10) in the vase water of cut 'Tiber' lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.602	1	1.602	33.138	0.005
Error	0.193	4	0.048		
Total	50.530	6			

Table B.2.37: ANOVA table for changes of water uptake of cut for 'Tiber' lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	0.000	1.000
Error	0.027	10	0.003		
Total	0.240	12			

Table B.2.38: ANOVA table for changes of water uptake of cut for 'Tiber' lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	0.738	0.411
Error	0.102	10	0.010		
Total	1.130	12			

Table B.2.39: ANOVA table for changes of water uptake of cut for 'Tiber' lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.001	1	0.001	0.122	0.734
Error	0.068	10	0.007		
Total	0.770	12			

Table B.2.40: ANOVA table for changes of water uptake of cut for 'Tiber' lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.013	1	0.013	1.818	0.207
Error	0.073	10	0.007		
Total	0.940	12			

Table B.2.41: ANOVA table for fresh weight of cut 'Tiber' lilies on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2.166	1	2.166	0.057	0.817
Error	303.435	8	37.929		
Total	132624.610	10			

Table B.2.42: ANOVA table for fresh weight of cut 'Tiber' lilies on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	13.728	1	13.728	0.170	0.691
Error	647.156	8	80.894		
Total	133694.040	10			

Table B.2.43: ANOVA table for fresh weight of cut 'Tiber' lilies on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	23.313	1	23.313	0.218	0.653
Error	856.623	8	107.078		
Total	118993.360	10			

Table B.2.44: ANOVA table for fresh weight of cut 'Tiber' lilies on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	60.401	1	60.401	0.427	0.532
Error	1131.983	8	141.498		
Total	83348.480	10			

Table B.2.45: ANOVA table for vase life of cut 'Tiber' lilies when placed as single and two stems per vase.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.521	1	0.521	0.676	0.430
Error	7.708	10	0.771		
Total	1538.250	12			

Experiment II: Bacteria population in vase water and the vase life of the cut 'Akito' rose.

Table B.2.46: ANOVA table for changes of total bacteria plate count of cut 'Akito' roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.215	1	1.215	2.119	0.219
Error	2.293	4	0.573		
Total	170.990	6			

Table B.2.47: ANOVA table for changes of total bacteria plate count of cut 'Akito' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.327	1	0.327	1.225	0.330
Error	1.067	4	0.267		
Total	257.500	6			

Table B.2.48: ANOVA table for changes of total bacteria plate count of cut 'Akito' roses s on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	0.009	0.927
Error	2.827	4	0.707		
Total	246.040	6			

Table B.2.49: ANOVA table for changes of total bacteria plate count of cut 'Akito' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.015	1	0.015	0.158	0.711
Error	0.380	4	0.095		
Total	242.330	6			

Table B.2.50: ANOVA table for changes of *Neisseria spp*. (A1) of cut 'Akito' roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.240	1	0.240	0.818	0.417
Error	1.173	4	0.293		
Total	100.640	6			

Table B.2.51: ANOVA table for changes of *Neisseria spp*. (A1) of cut 'Akito' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.707	1	1.707	5.389	0.081
Error	1.267	4	0.317		
Total	169.400	6			

Table B.2.52: ANOVA table for changes of *Neisseria spp*. (A1) of cut 'Akito' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.707	1	1.707	4.697	0.096
Error	1.453	4	0.363		
Total	178.120	6			

Table B.2.53: ANOVA table for changes of *Neisseria spp*. (A1) of cut 'Akito' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.027	1	0.027	0.348	0.587
Error	0.307	4	0.077		
Total	264.340	6			

Table B.2.54: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (A36) of cut 'Akito' roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.540	1	0.540	0.824	0.415
Error	2.620	4	0.655		
Total	178.120	6			

Table B.2.55: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (A36) of cut 'Akito' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	0.571	0.492
Error	0.047	4	0.012		
Total	280.220	6			

Table B.2.56: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (A36) of cut 'Akito' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.042	1	0.042	1.923	0.238
Error	0.087	4	0.022		
Total	262.810	6			

Table B.2.57: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (A36) of cut 'Akito' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	0.045	0.842
Error	0.587	4	0.147		
Total	256.700	6			

Table B.2.58: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (A55) of cut 'Akito' roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.202	1	0.202	30.250	0.005
Error	0.027	4	0.007		
Total	37.230	6			

Table B.2.59: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (A55) of cut 'Akito' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.027	1	0.027	4.000	0.116
Error	0.027	4	0.007		
Total	114.460	6			

Table B.2.60: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (A55) of cut 'Akito' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.027	1	0.027	4.000	0.116
Error	0.027	4	0.007		
Total	142.160	6			

Table B.2.61: ANOVA table for changes of *Streptococcus spp.* or *Enterococcus spp.* (A55) of cut 'Akito' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	-	-
Error	0.000	4	0.000		
Total	138.240	6			

Table B.2.62: ANOVA table for changes of water uptake rate of cut 'Akito' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	0.616	0.451
Error	0.122	10	0.012		
Total	9.670	12			

Table B.2.63: ANOVA table for changes of water uptake rate of cut 'Akito' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.008	1	0.008	0.918	0.360
Error	0.082	10	0.008		
Total	5.290	12			

Table B.2.64: ANOVA table for changes of water uptake rate of cut 'Akito' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.120	1	0.120	10.909	0.008
Error	0.110	10	0.011		
Total	3.860	12			

Table B.2.65: ANOVA table for changes of fresh weight of cut 'Akito' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	138.720	1	138.720	14.274	0.004
Error	97.187	10	9.719		
Total	128121.360	12			

Table B.2.66: ANOVA table for changes of fresh weight of cut 'Akito' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	249.341	1	249.341	14.199	0.004
Error	175.608	10	17.561		
Total	113197.190	12			

Table B.2.67: ANOVA table for changes of fresh weight of cut 'Akito' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	329.701	1	329.701	6.224	0.032
Error	529.702	10	52.970		
Total	90179.910	12			

Table B.2.68: ANOVA table for vase life of cut 'Akito' roses when placed as a single and two stems per vase.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	9.013	1	9.013	4.124	0.070
Error	21.853	10	2.185		
Total	1222.880	12			

Experiment III: Bacteria population in vase water and the vase life of cut 'Valentino' rose.

Table B.2.69: ANOVA table for changes of total bacteria plate count of cut 'Valentino' roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.027	1	0.027	16.000	0.016
Error	0.007	4	0.002		
Total	166.460	6			

Table B.2.70: ANOVA table for changes of total bacteria plate count of cut 'Valentino' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.602	1	0.602	18.050	0.013
Error	0.133	4	0.033		
Total	250.350	6			

Table B.2.71: ANOVA table for changes of total bacteria plate count of cut 'Valentino' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.135	1	0.135	0.271	0.630
Error	1.993	4	0.498		
Total	189.170	6			

Table B.2.72: ANOVA table for changes of total bacteria plate count of cut 'Valentino' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.082	1	0.082	0.239	0.651
Error	1.367	4	0.342		
Total	158.530	6			

Table B.2.73: ANOVA table for changes of *Pseudomonas spp*. (V1) of cut 'Valentino' roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.003	1	0.003	0.588	0.461
Error	0.057	10	0.006		
Total	164.340	12			

Table B.2.74: ANOVA table for changes of *Pseudomonas spp*. (V1) of cut 'Valentino' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.367	1	0.367	49.000	0.000
Error	0.075	10	0.007		
Total	262.710	12			

Table B.2.75: ANOVA table for changes of *Pseudomonas spp*. (V1) of cut 'Valentino' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	0.000	1.000
Error	2.427	10	0.243		
Total	275.080	12			

Table B.2.76: ANOVA table for changes of *Pseudomonas spp*. (V1) of cut 'Valentino' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.203	1	1.203	8.376	0.016
Error	1.437	10	0.144		
Total	194.640	12			

Table B.2.77: ANOVA table for changes of *Bacillus spp*. (V20) of cut 'Valentino' roses on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.213	1	0.213	8.767	0.014
Error	0.243	10	0.024		
Total	122.060	12			

Table B.2.78: ANOVA table for changes of *Bacillus spp*. (V20) of cut 'Valentino' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.120	1	0.120	1.875	0.201
Error	0.640	10	0.064		
Total	131.440	12			

Table B.2.79: ANOVA table for changes of *Bacillus spp*. (V20) of cut 'Valentino' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.853	1	0.853	5.356	0.043
Error	1.593	10	0.159		
Total	138.460	12			

Table B.2.80: ANOVA table for changes of *Bacillus spp*. (V20) of cut 'Valentino' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.053	1	0.053	0.121	0.735
Error	4.403	10	0.440		
Total	194.860	12			

Table B.2.81: ANOVA table for changes of *Staphylococcus spp.* (V45) of cut 'Valentino' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.653	1	0.653	8.909	0.014
Error	0.733	10	0.073		
Total	187.040	12			

Table B.2.82: ANOVA table for changes of *Staphylococcus spp.* (V45) of cut 'Valentino' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3.521	1	3.521	38.761	0.000
Error	0.908	10	0.091		
Total	317.570	12			

Table B.2.83: ANOVA table for changes of *Staphylococcus spp.* (V45) of cut 'Valentino' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.907	1	0.907	5.292	0.044
Error	1.715	10	0.172		
Total	248.330	12			

Table B.2.84: ANOVA table for changes of water uptake rate of cut 'Valentino' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	0.170	0.689
Error	0.442	10	0.044		
Total	7.970	12			

Table B.2.85: ANOVA table for changes of water uptake rate of cut 'Valentino' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.083	1	0.083	3.289	0.100
Error	0.253	10	0.025		
Total	4.420	12			

Table B.2.86: ANOVA table for changes of water uptake rate of cut 'Valentino' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.053	1	0.053	3.019	0.113
Error	0.177	10	0.018		
Total	3.860	12			

Table B.2.87: ANOVA table for changes of fresh weight of cut 'Valentino' roses on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	13.021	1	13.021	0.215	0.653
Error	605.868	10	60.587		
Total	147031.410	12			

Table B.2.88: ANOVA table for changes of fresh weight of cut 'Valentino' roses on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	56.333	1	56.333	1.512	0.247
Error	372.493	10	37.249		
Total	131137.640	12			

Table B.2.89: ANOVA table for changes of fresh weight of cut 'Valentino' roses on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	116.563	1	116.563	4.428	0.062
Error	263.217	10	26.322		
Total	103054.780	12			

Table B.2.90: ANOVA table for vase life of cut 'Valentino' roses which were placed as a single and two stems per vase.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3.413	1	3.413	2.242	0.165
Error	15.227	10	1.523		
Total	1171.120	12			

Experiment IV: Bacteria population in vase water and the vase life of a mixed flowers bouquet held in distilled water.

Table B.2.91: ANOVA table for changes of bacteria plate count of mixed flowers bouquets on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.002	1	0.002	0.250	0.643
Error	0.027	4	0.007		
Total	126.070	6			

Table B.2.92: ANOVA table for changes of bacteria plate count of mixed flowers bouquets on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.202	1	0.202	17.286	0.014
Error	0.047	4	0.012		
Total	222.290	6			

Table B.2.93: ANOVA table for changes of bacteria plate count of mixed flowers bouquets on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.202	1	0.202	3.903	0.119
Error	0.207	4	0.052		
Total	183.010	6			

Table B.2.94: ANOVA table for changes of bacteria plate count of mixed flowers bouquets on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.807	1	0.807	69.143	0.001
Error	0.047	4	0.012		
Total	205.020	6			

Table B.2.95: ANOVA table for changes of bacteria plate count of mixed flowers bouquets on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.815	1	1.815	24.750	0.008
Error	0.293	4	0.073		
Total	233.990	6			

Table B.2.96: ANOVA table for changes of *Neisseria spp*. (LA18) in the vase water of mixed flowers bouquets held in distilled water on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.270	1	0.270	7.500	0.021
Error	0.360	10	0.036		
Total	119.700	12			

Table B.2.97: ANOVA table for changes of *Neisseria spp*. (LA18) in the vase water of mixed flowers bouquets held in distilled water on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.270	1	0.270	7.500	0.021
Error	0.360	10	0.036		
Total	119.700	12			

Table B.2.98: ANOVA table for changes of *Neisseria spp*. (LA18) in the vase water of mixed flowers bouquets held in distilled water on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.301	1	0.301	4.688	0.056
Error	0.642	10	0.064		
Total	268.850	12			

Table B.2.99: ANOVA table for changes of *Neisseria spp*. (LA18) in the vase water of mixed flowers bouquets held in distilled water on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	4.813	1	4.813	34.463	0.000
Error	1.397	10	0.140		
Total	254.640	12			

Table B.2.100: ANOVA table for changes of *Neisseria spp*. (LA18) in the vase water of mixed flowers bouquets held in distilled water on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.053	1	0.053	0.270	0.614
Error	1.973	10	0.197		
Total	294.080	12			

Table B.2.101: ANOVA table for changes of *Neisseria spp*. (LA45) in the vase water of mixed flowers bouquets held in distilled water on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	-	-
Error	0.000	10	0.000		
Total	108.000	12			

Table B.2.102: ANOVA table for changes of *Neisseria spp*. (LA45) in the vase water of mixed flowers bouquets held in distilled water on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	-	-
Error	0.000	10	0.000		
Total	300.000	12			

Table B.2.103: ANOVA table for changes of *Neisseria spp*. (LA45) in the vase water of mixed flowers bouquets held in distilled water on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.030	1	0.030	0.625	0.448
Error	0.480	10	0.048		
Total	217.260	12			

Table B.2.104: ANOVA table for changes of *Neisseria spp*. (LA45) in the vase water of mixed flowers bouquets held in distilled water on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.920	1	1.920	40.000	0.000
Error	0.480	10	0.048		
Total	256.320	12			

Table B.2.105: ANOVA table for changes of *Neisseria spp*. (LA45) in the vase water of mixed flowers bouquets held in distilled water on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.841	1	1.841	97.743	0.000
Error	0.188	10	0.019		
Total	273.730	12			

Table B.2.106: ANOVA table for changes of water uptake of cut mixed flowers bouquets held in distilled water on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.003	1	0.003	1.250	0.290
Error	0.027	10	0.003		
Total	1.500	12			

Table B.2.105: ANOVA table for changes of water uptake of cut mixed flowers bouquets held in distilled water on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.001	1	0.001	1.000	0.341
Error	0.008	10	0.001		
Total	1.150	12			

Table B.2.108: ANOVA table for changes of water uptake of cut mixed flowers bouquets held in distilled water on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.021	1	0.021	25.000	0.001
Error	0.008	10	0.001		
Total	0.730	12			

Table B.2.109: ANOVA table for changes of water uptake of cut mixed flowers bouquets held in distilled water on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.041	1	0.041	49.000	0.000
Error	0.008	10	0.001		
Total	0.350	12			

Table B.2.110: ANOVA table for changes of the fresh weight of 'Tiber' lily in mixed flowers bouquets held in distilled water on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3.968	1	3.968	1.077	0.324
Error	36.842	10	3.684		
Total	153924.910	12			

Table B.2.111: ANOVA table for changes of the fresh weight of 'Tiber' lily in mixed flowers bouquets held in distilled water on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	108.000	1	108.000	21.366	0.001
Error	50.547	10	5.055		
Total	162559.880	12			

Table B.2.112: ANOVA table for changes of the fresh weight of 'Tiber' lily in mixed flowers bouquets held in distilled water on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	222.741	1	222.741	12.315	0.006
Error	180.868	10	18.087		
Total	135384.050	12			

Table B.2.113: ANOVA table for changes of the fresh weight of 'Tiber' lily in mixed flowers bouquets held in distilled water on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	245.707	1	245.707	4.503	0.060
Error	545.622	10	54.562		
Total	91011.350	12			

Table B.2.114: ANOVA table for changes of the fresh weight of 'Akito' rose in mixed flowers bouquets held in distilled water on day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	-	-
Error	0.000	10	0.000		
Total	120000.000	12			

Table B.2.115: ANOVA table for changes of the fresh weight of 'Akito' rose in mixed flowers bouquets held in distilled water on day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	65.801	1	65.801	7.759	0.019
Error	84.808	10	8.481		
Total	131172.710	12			

Table B.2.116: ANOVA table for changes of the fresh weight of 'Akito' rose in mixed flowers bouquets held in distilled water on day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	332.853	1	332.853	18.729	0.001
Error	177.723	10	17.772		
Total	113845.780	12			

Table B.2.117: ANOVA table for changes of the fresh weight of 'Akito' rose in mixed flowers bouquets held in distilled water on day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1573.230	1	1573.230	58.474	0.000
Error	269.050	10	26.905		
Total	90800.800	12			

Table B.2.118: ANOVA table for changes of the fresh weight of 'Akito' rose in mixed flowers bouquets held in distilled water on day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3017.841	1	3017.841	96.046	0.000
Error	314.208	10	31.421		
Total	69741.490	12			

Table B.2.119: ANOVA table for vase life of 'Tiber' lily in mixed flowers bouquets held in distilled water.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	4.083	1	4.083	17.500	0.002
Error	2.333	10	0.233		
Total	1436.500	12			

Table B.2.120: ANOVA table for vase life of 'Akito' rose in mixed flowers bouquets held in distilled water.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	44.853	1	44.853	69.938	0.000
Error	6.413	10	0.641		
Total	1149.520	12			

Experiment V: Bacterial population in vase water and the vase life of mixed flowers bouquets held in flower food.

Table B.2.121: ANOVA Table for changes of bacteria plate count of flowers in mixed flowers bouquets held in flower food at day 0

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	0.000	1.000
Error	0.053	4	0.013		
Total	97.660	6			

Table B.2.122: ANOVA Table for changes of bacteria plate count of flowers in mixed flowers bouquets held in flower food at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.135	1	0.135	4.500	0.101
Error	0.120	4	0.030		
Total	63.630	6			

Table B.2.123: ANOVA Table for changes of bacteria plate count of flowers in mixed flowers bouquets held in flower food at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	4.000	0.116
Error	0.007	4	0.002		
Total	35.540	6			

Table B.2.124: ANOVA Table for changes of bacteria plate count of flowers in mixed flowers bouquets held in flower food at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.002	1	0.002	0.053	0.830
Error	0.127	4	0.032		
Total	28.730	6			

Table B.2.125: ANOVA Table for changes of bacteria plate count of flowers in mixed flowers bouquets held in flower food at day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.282	1	0.282	3.018	0.157
Error	0.373	4	0.093		
Total	23.470	6			

Table B.2.126: ANOVA Table for *Staphylococcus spp.* (LAF8) in mixed flowers bouquets held in flower food at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.333	1	0.333	19.231	0.001
Error	0.173	10	0.017		
Total	29.960	12			

Table B.2.127: ANOVA Table for *Staphylococcus spp.* (LAF8) in mixed flowers bouquets held in flower food at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	0.172	0.687
Error	0.435	10	0.044		
Total	19.950	12			

Table B.2.128: ANOVA Table for *Staphylococcus spp.* (LAF10) in mixed flowers bouquets held in flower food at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.333	1	0.333	4.032	0.072
Error	0.827	10	0.083		
Total	54.080	12			

Table B.2.129: ANOVA Table for *Staphylococcus spp.* (LAF10) in mixed flowers bouquets held in flower food at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.083	1	0.083	1.359	0.271
Error	0.613	10	0.061		
Total	49.500	12			

Table B.2.130: ANOVA Table for *Staphylococcus spp.* (LAF10) in mixed flowers bouquets held in flower food at day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.853	1	0.853	320.000	0.000
Error	0.027	10	0.003		
Total	44.200	12			

Table B.2.131: ANOVA Table for *Neisseria spp*. (LAF13) in mixed flowers bouquets held in flower food at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.003	1	0.003	0.042	0.842
Error	0.793	10	0.079		
Total	107.600	12			

Table B.2.132: ANOVA Table for *Neisseria spp*. (LAF13) in mixed flowers bouquets held in flower food at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.030	1	0.030	2.500	0.145
Error	0.120	10	0.012		
Total	50.580	12			

Table B.2.133: ANOVA Table for *Neisseria spp*. (LAF18) in mixed flowers bouquets held in flower food at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.003	1	0.003	0.250	0.628
Error	0.133	10	0.013		
Total	52.220	12			

Table B.2.134: ANOVA Table for *Neisseria spp.* (LAF18) in mixed flowers bouquets held in flower food at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	0.556	0.473
Error	0.135	10	0.014		
Total	49.350	12			

Table B.2.135: ANOVA Table for *Neisseria spp*. (LAF18) in mixed flowers bouquets held in flower food at day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.203	1	1.203	8.936	0.014
Error	1.347	10	0.135		
Total	43.620	12			

Table B.2.136: ANOVA Table for water uptake of flowers in mixed flowers bouquets held in flower food at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.013	1	0.013	5.000	0.049
Error	0.027	10	0.003		
Total	1.120	12			

Table B.2.137: ANOVA Table for water uptake of flowers in mixed flowers bouquets held in flower food at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	1	0.007	5.000	0.049
Error	0.015	10	0.001		
Total	0.630	12			

Table B.2.138: ANOVA Table for water uptake of flowers in mixed flowers bouquets held in flower food at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.001	1	0.001	0.385	0.549
Error	0.022	10	0.002		
Total	0.630	12			

Table B.2.139: ANOVA Table for water uptake of flowers in mixed flowers bouquets held in flower food at day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.001	1	0.001	0.294	0.599
Error	0.028	10	0.003		
Total	0.470	12			

Table B.2.140: ANOVA Table for fresh weight of 'Tiber' lilies in mixed flowers bouquets held in flower food at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	-	-
Error	0.000	10	0.000		
Total	120000.000	12			

Table B.2.141: ANOVA Table for fresh weight of 'Tiber' lilies in mixed flowers bouquets held in flower food at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.563	1	0.563	0.173	0.687
Error	32.633	10	3.263		
Total	148640.960	12			

Table B.2.142: ANOVA Table for fresh weight of 'Tiber' lilies in mixed flowers bouquets held in flower food at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	5.070	1	5.070	0.219	0.650
Error	231.477	10	23.148		
Total	145876.880	12			

Table B.2.143: ANOVA Table for fresh weight of 'Tiber' lilies in mixed flowers bouquets held in flower food at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	17.280	1	17.280	0.186	0.675
Error	929.540	10	92.954		
Total	112925.540	12			

Table B.2.144: ANOVA Table for fresh weight of 'Tiber' lilies in mixed flowers bouquets held in flower food at day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	4.813	1	4.813	0.034	0.857
Error	1399.143	10	139.914		
Total	72891.160	12			

Table B.2.145: ANOVA Table for fresh weight of 'Akito' roses in mixed flowers bouquets held in flower food at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	1	0.000	-	-
Error	0.000	10	0.000		
Total	120000.000	12			

Table B.2.146: ANOVA Table for fresh weight of 'Akito' roses in mixed flowers bouquets held in flower food at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.053	1	0.053	0.038	0.849
Error	13.917	10	1.392		
Total	141932.720	12			

Table B.2.147: ANOVA Table for fresh weight of 'Akito' roses in mixed flowers bouquets held in flower food at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	9.363	1	9.363	0.662	0.435
Error	141.433	10	14.143		
Total	132913.200	12			

Table B.2.148: ANOVA Table for fresh weight of 'Akito' roses in mixed flowers bouquets held in flower food at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	66.741	1	66.741	2.432	0.150
Error	274.468	10	27.447		
Total	119722.010	12			

Table B.2.149: ANOVA Table for fresh weight of 'Akito' roses in mixed flowers bouquets held in flower food at day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	192.000	1	192.000	6.378	0.030
Error	301.027	10	30.103		
Total	105250.480	12			

Table B.2.150: ANOVA Table for vase of 'Tiber' lilies in mixed flowers bouquets held in flower food.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.021	1	1.021	1.644	0.229
Error	6.208	10	0.621		
Total	1514.750	12			

Table B.2.151: ANOVA Table for vase life of 'Akito' roses in mixed flowers bouquets held in flower food.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	5.201	1	5.201	13.875	0.004
Error	3.748	10	0.375		
Total	1419.450	12			

B.3 ANOVA tables for chapter 4

Experiment I: Investigation of chemical exudates from 'Akito' rose stems on the vase life of the 'Tiber' lily

Table B.3.1: ANOVA Table for changes of water uptake of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water at day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.002	2	0.001	2.251	0.152
Error	0.006	11	0.001		
Total	0.314	14			

Table B.3.2: ANOVA Table for changes of water uptake of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.004	2	0.002	3.348	0.073
Error	0.007	11	0.001		
Total	0.215	14			

Table B.3.3: ANOVA Table for changes of water uptake of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water at day 5.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.002	2	0.001	1.807	0.210
Error	0.006	11	0.001		
Total	0.074	14			

Table B.3.4: ANOVA Table for changes of water uptake of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water at day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.440	2	0.220	2.771	0.106
Error	0.874	11	0.079		
Total	50.196	14			

Table B.3.5: ANOVA Table for changes of water uptake of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	2	0.003	5.063	0.028
Error	0.007	11	0.001		
Total	0.160	14			

Table B.3.6: ANOVA Table for changes of water uptake of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water at day 11.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.008	2	0.004	3.877	0.053
Error	0.011	11	0.001		
Total	0.154	14			

Table B.3.7: ANOVA Table for changes of fresh weight of the 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase water at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	2	0.000	-	-
Error	0.000	11	0.000		
Total	140000.000	14			

Table B.3.8: ANOVA Table for changes of fresh weight of the 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase water at day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	10.029	2	5.015	7.826	0.008
Error	7.048	11	0.641		
Total	149947.180	14			

Table B.3.9: ANOVA Table for changes of fresh weight of the 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase water at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	62.353	2	31.176	5.061	0.028
Error	67.762	11	6.160		
Total	164486.230	14			

Table B.3.10: ANOVA Table for changes of fresh weight of the 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase water at day 5.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	85.812	2	42.906	3.062	0.088
Error	154.152	11	14.014		
Total	168651.410	14			

Table B.3.11: ANOVA Table for changes of fresh weight of the 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase water at day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	81.424	2	40.712	1.449	0.276
Error	309.170	11	28.106		
Total	153399.520	14			

Table B.3.12: ANOVA Table for changes of fresh weight of the 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase water at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	92.786	2	46.393	1.215	0.334
Error	420.131	11	38.194		
Total	130845.820	14			

Table B.3.13: ANOVA Table for changes of fresh weight of the 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase water at day 11.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	123.190	2	61.595	1.273	0.318
Error	532.084	11	48.371		
Total	104302.500	14			

Table B.3.14: ANOVA Table for time to opening of the primary bud 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.929	2	0.464	0.851	0.453
Error	6.000	11	0.545		
Total	523.000	14			

Table B.3.14: ANOVA Table for vase life of 'Tiber' lily held in sterile distilled water, sterile vase water and non-sterile vase.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2.850	2	1.425	3.044	0.089
Error	5.150	11	0.468		
Total	1702.000	14			

Table B.3.16: ANOVA Table for total bacterial plate count of the 'Tiber' lily held in sterile distilled water, sterile old water and non-sterile old water at day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.447	2	0.723	130.200	0.000
Error	0.033	6	0.006		
Total	405.490	9			

Experiment II: Investigation into chemical exudates from 'Tiber' lily stems on the vase life of 'Akito' rose.

Table B.3.17: ANOVA Table for changes of water uptake of the 'Akito' rose held in sterile distilled water, sterile old water and non-sterile old water at day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.005	2	0.002	0.184	0.835
Error	0.116	9	0.013		
Total	2.587	12			

Table B.3.18: ANOVA Table for changes of water uptake of the 'Akito' rose held in sterile distilled water, sterile old water and non-sterile old water at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	2	0.003	0.324	0.731
Error	0.094	9	0.010		
Total	3.988	12			

Table B.3.19: ANOVA Table for changes of water uptake of the 'Akito' rose held in sterile distilled water, sterile old water and non-sterile old water at day 5.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.001	2	0.000	0.118	0.890
Error	0.031	9	0.003		
Total	1.179	12			

Table B.3.20: ANOVA Table for changes of water uptake of the 'Akito' rose held in sterile distilled water, sterile old water and non-sterile old water at day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.001	2	0.000	0.098	0.908
Error	0.043	9	0.005		
Total	1.599	12			

Table B.3.21: ANOVA Table for changes of water uptake of the 'Akito' rose held in sterile distilled water, sterile old water and non-sterile old water at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.001	2	0.001	0.124	0.885
Error	0.047	9	0.005		
Total	1.123	12			

Table B.3.22: ANOVA Table for changes of water uptake of the 'Akito' rose held in sterile distilled water, sterile old water and non-sterile old water at day 11.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.001	2	0.000	0.056	0.946
Error	0.041	9	0.005		
Total	0.927	12			

Table B.3.23: ANOVA Table for changes of the fresh weight of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	2	0.000	-	-
Error	0.000	9	0.000		
Total	120000.000	12			

Table B.3.24: ANOVA Table for changes of the fresh weight of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water at day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	52.820	2	26.410	12.928	0.002
Error	18.386	9	2.043		
Total	133549.820	12			

Table B.3.25: ANOVA Table for changes of the fresh weight of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	134.125	2	67.062	7.217	0.013
Error	83.625	9	9.292		
Total	126190.270	12			

Table B.3.26: ANOVA Table for changes of the fresh weight of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water at day 5.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	91.921	2	45.960	3.630	0.070
Error	113.949	9	12.661		
Total	127658.110	12			

Table B.3.27: ANOVA Table for changes of the fresh weight of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water at day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	29.410	2	14.705	0.785	0.485
Error	168.659	9	18.740		
Total	121984.670	12			

Table B.3.28: ANOVA Table for changes of the fresh weight of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	17.058	2	8.529	0.350	0.714
Error	219.365	9	24.374		
Total	118145.610	12			

Table B.3.29: Table for changes of the fresh weight of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water at day 11.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	12.327	2	6.164	0.205	0.819
Error	271.182	9	30.131		
Total	109173.310	12			

Table B.3.30: B.3.1 ANOVA Table for final stage of bud opening and vase life of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.200	2	0.600	0.835	0.465
Error	6.467	9	0.719		
Total	216.000	12			

Table B.3.31: ANOVA Table for vase life of the 'Akito' rose held in sterile distilled water, sterile vase water and non-sterile vase water.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.367	2	0.683	0.315	0.738
Error	19.550	9	2.172		
Total	1365.000	12			

Table B.3.32: ANOVA Table for total bacteria plate count of the 'Akito' rose held in sterile distilled water, sterile old water and non-sterile old water at day 12.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.460	2	0.730	-	-
Error	0.000	6	0.000		
Total	385.620	9			

B.4 ANOVA tables for chapter 5

Experiment I: Effects of essential oils on bacteria growth of cut 'Tiber' lily.

Table B.4.1: ANOVA Table for zone of inhibition of L4 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2192.359	12	182.697	52.779	0.000
Error	90.000	26	3.462		
Total	5532.000	39			

Table B.4.2: ANOVA Table for zone of inhibition of L7 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	249.641	12	20.803	8.364	0.000
Error	64.667	26	2.487		
Total	1578.000	39			

Table B.4.3: ANOVA Table zone of inhibition of L9 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1371.897	12	114.325	20.359	0.000
Error	146.000	26	5.615		
Total	4482.000	39			

Table B.4.4: ANOVA Table for zone of inhibition of L15 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	95.641	12	7.970	2.852	0.012
Error	72.667	26	2.795		
Total	1267.000	39			

Table B.4.5: ANOVA Table for zone of inhibition of L29 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	83.231	12	6.936	2.818	0.013
Error	64.000	26	2.462		
Total	897.000	39			

Table B.4.6: ANOVA Table for zone of inhibition of A1 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2338.974	12	194.915	46.352	0.000
Error	109.333	26	4.205		
Total	6592.000	39			

Table B.4.7: ANOVA Table for zone of inhibition of A36 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2579.026	12	214.919	43.205	0.000
Error	129.333	26	4.974		
Total	7538.000	39			

Table B.4.8: ANOVA Table for zone of inhibition of A55 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	5815.744	12	484.645	461.004	0.000
Error	27.333	26	1.051		
Total	14439.000	39			

Table B.4.9: ANOVA Table for zone of inhibition of V1 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1818.410	12	151.534	40.478	0.000
Error	97.333	26	3.744		
Total	4301.000	39			

Table B.4.10: ANOVA Table for zone of inhibition of V20 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3049.590	12	254.132	119.412	0.000
Error	55.333	26	2.128		
Total	11612.000	39			

Table B.4.11: ANOVA Table for zone of inhibition of V45 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2870.769	12	239.231	85.596	0.000
Error	72.667	26	2.795		
Total	8322.000	39			

Table B.4.12: ANOVA Table for zone of inhibition of LA15 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2889.692	12	240.808	93.915	0.000
Error	66.667	26	2.564		
Total	6957.000	39			

Table B.4.13: ANOVA Table for zone of inhibition of LA18 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3459.641	12	288.303	130.742	0.000
Error	57.333	26	2.205		
Total	8258.000	39			

Table B.4.14: ANOVA Table for zone of inhibition of LA45 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2010.769	12	167.564	79.695	0.000
Error	54.667	26	2.103		
Total	4943.000	39			

Table B.4.15: ANOVA Table for zone of inhibition of LAF8 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3535.077	12	294.590	122.223	0.000
Error	62.667	26	2.410		
Total	10008.000	39			

Table B.4.16: ANOVA Table for zone of inhibition of LAF10 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3594.974	12	299.581	307.465	0.000
Error	25.333	26	0.974		
Total	9903.000	39			

Table B.4.17: ANOVA Table zone of inhibition of LAF13 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3631.231	12	302.603	125.548	0.000
Error	62.667	26	2.410		
Total	11033.000	39			

Table B.4.18: ANOVA Table for zone of inhibition of LAF18 when exposed to various essential oils diluted 1:10 methanol after 48 h at 37°C.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3599.897	12	299.991	95.119	0.000
Error	82.000	26	3.154		
Total	10221.000	39			

Experiment III: The effect of thyme oil on the vase life of the 'Tiber' lily

Table B.4.19: ANOVA Table for bacteria plate count in the vase water of 'Tiber' lilies at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	3.563	3	1.188	21.928	0.000
Error	0.433	8	0.054		
Total	113.200	12			

Table B.4.20: ANOVA Table for bacteria plate count in the vase water of 'Tiber' lilies at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	53.665	8	6.708	45.167	0.000
Error	2.673	18	0.149		
Total	601.290	27			

Table B.4.21: ANOVA Table for bacteria plate count in the vase water of 'Tiber' lilies at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	7.287	7	1.041	16.545	0.000
Error	1.007	16	0.063		
Total	867.500	24			

Table B.4.22: ANOVA Table for bacteria plate count in the vase water of 'Tiber' lilies at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	11.167	7	1.595	27.946	0.000
Error	0.913	16	0.057		
Total	979.820	24			

Table B.4.23: ANOVA Table for changes of pH in the vase water of 'Tiber' lilies at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	11.853	8	1.482	17.905	0.000
Error	1.490	18	0.083		
Total	613.539	27			

Table B.4.24: ANOVA Table for changes of pH in the vase water of 'Tiber' lilies at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	9.951	8	1.244	72.273	0.000
Error	0.310	18	0.017		
Total	567.683	27			

Table B.4.25: ANOVA Table for changes of pH in the vase water of 'Tiber' lilies at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	12.851	8	1.606	6.627	0.000
Error	4.363	18	0.242		
Total	639.390	27			

Table B.4.26: ANOVA Table for changes of pH in the vase water of 'Tiber' lilies at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	9.383	8	1.173	19.105	0.000
Error	1.105	18	0.061		
Total	554.272	27			

Table B.4.27: ANOVA Table for changes in water uptake rate of the 'Tiber' lilies at day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.015	8	0.002	4.181	0.006
Error	0.008	18	0.000		
Total	2.514	27			

Table B.4.28: ANOVA Table for changes in water uptake rate of the 'Tiber' lilies at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	8	0.001	5.289	0.002
Error	0.003	18	0.000		
Total	0.585	27			

Table B.4.29: ANOVA Table for changes in water uptake rate of the 'Tiber' lilies at day 5.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.005	8	0.001	2.071	0.095
Error	0.005	18	0.000		
Total	0.365	27			

Table B.4.30: ANOVA Table for changes in water uptake rate of the 'Tiber' lilies at day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.007	8	0.001	1.594	0.195
Error	0.010	18	0.001		
Total	0.860	27			

Table B.4.31: ANOVA Table for changes in water uptake rate of the 'Tiber' lilies at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.016	8	0.002	5.195	0.002
Error	0.007	18	0.000		
Total	0.490	27			

Table B.4.32: ANOVA Table for changes in water uptake rate of the 'Tiber' lilies at day 11.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.008	2	0.004	3.800	0.086
Error	0.006	6	0.001		
Total	0.382	9			

Table B.4.33: ANOVA Table for changes of the fresh weight of the 'Tiber' lilies at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	8	0.000	-	-
Error	0.000	18	0.000		
Total	270000.000	27			

Table B.4.34: ANOVA Table for changes of the fresh weight of the 'Tiber' lilies at day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	11.933	8	1.492	1.909	0.121
Error	14.067	18	0.781		
Total	317681.480	27			

Table B.4.35: ANOVA Table for changes of the fresh weight of the 'Tiber' lilies at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	27.776	8	3.472	1.150	0.379
Error	54.327	18	3.018		
Total	361285.970	27			

Table B.4.36: ANOVA Table for changes of the fresh weight of the 'Tiber' lilies at day 5.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	836.007	8	104.501	5.633	0.001
Error	333.953	18	18.553		
Total	364064.480	27			

Table B.4.37: ANOVA Table for changes of the fresh weight of the 'Tiber' lilies at day 7.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1480.080	8	185.010	7.051	0.000
Error	472.320	18	26.240		
Total	339029.520	27			

Table B.4.38: ANOVA Table for changes of the fresh weight of the 'Tiber' lilies at day 9.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1303.803	8	162.975	3.293	0.017
Error	890.967	18	49.498		
Total	288658.370	27			

Table B.4.39: ANOVA Table for changes of the fresh weight of the 'Tiber' lilies at day 11.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	234.749	2	117.374	2.736	0.143
Error	257.353	6	42.892		
Total	88266.040	9			

Table B.4.40: ANOVA Table for vase lives of 'Tiber' lilies held in distilled water, Chrysal, 5% glycerol, 0.78 to 25 mg/ mL thyme oil.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	26.148	8	3.269	18.010	0.000
Error	8.167	45	0.181		
Total	5061.000	54			

Experiment III: The effect of thyme oil on the vase life of the 'Akito' rose.

Table B.4.41: ANOVA Table for the vase lives of 'Akito' roses held in distilled water, Chrysal, 5% glycerol, 0.78 to 25 mg/ mL thyme oil.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	809.704	8	101.213	130.131	0.000
Error	98.000	126	0.778		
Total	3664.000	135			

Table B.4.42: ANOVA Table for total bacterial plate count in the vase water of the 'Akito' roses at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	47.603	8	5.950	32.855	0.000
Error	3.260	18	0.181		
Total	287.900	27			

Table B.4.43: ANOVA Table for total bacterial plate count in the vase water of the 'Akito' roses at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	6.046	7	0.864	259.125	0.000
Error	0.053	16	0.003		
Total	780.170	24			

Table B.4.44: ANOVA Table for total bacterial plate count in the vase water of the 'Akito' roses at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	32.502	3	10.834	82.809	0.000
Error	1.047	8	0.131		
Total	436.070	12			

Table B.4.45: ANOVA Table for changes in pH in the vase water of the 'Akito' roses at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	11.087	8	1.386	59.140	0.000
Error	0.422	18	0.023		
Total	503.968	27			

Table B.4.46: ANOVA Table for changes in pH in the vase water of the 'Akito' roses at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	11.071	8	1.384	40.552	0.000
Error	0.614	18	0.034		
Total	579.331	27			

Table B.4.47: ANOVA Table for changes in pH in the vase water of the ‘Akito’ roses se at day 6.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	5.918	3	1.973	14.807	0.001
Error	1.066	8	0.133		
Total	252.240	12			

Table B.4.48: ANOVA Table for changes in water uptake rate of the ‘Akito’ roses at day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.219	8	0.027	6.146	0.001
Error	0.080	18	0.004		
Total	7.250	27			

Table B.4.49: ANOVA Table for changes in water uptake rate of the ‘Akito’ roses at day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.787	8	0.098	11.543	0.000
Error	0.153	18	0.009		
Total	4.570	27			

Table B.4.50: ANOVA Table for changes in water uptake rate of the ‘Akito’ roses at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1.847	8	0.231	56.659	0.000
Error	0.073	18	0.004		
Total	5.550	27			

Table B.4.51: ANOVA Table for changes in water uptake rate of the ‘Akito’ roses at day 5.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.407	3	0.136	32.533	0.000
Error	0.033	8	0.004		
Total	1.520	12			

Table B.4.52: ANOVA Table for changes in fresh weight of the 'Akito' roses at day 0.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	0.000	8	0.000	-	-
Error	0.000	18	0.000		
Total	270000.000	27			

Table B.4.53: ANOVA Table for changes in fresh weight of the 'Akito' roses at day 1.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	941.967	8	117.746	31.334	0.000
Error	67.640	18	3.758		
Total	355948.810	27			

Table B.4.54: ANOVA Table changes in fresh weight of the 'Akito' roses at day 2.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1146.753	8	143.344	31.338	0.000
Error	82.333	18	4.574		
Total	350617.900	27			

Table B.4.55: ANOVA Table for changes in fresh weight of the 'Akito' roses at day 3.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	1343.353	8	167.919	22.269	0.000
Error	135.727	18	7.540		
Total	328773.350	27			

Table B.4.56: ANOVA Table for changes in fresh weight of the 'Akito' roses at day 5.

Source of variation	s.s.	df	m.s.	F	Sig.
Treatments	2136.016	3	712.005	42.926	0.000
Error	132.693	8	16.587		
Total	134673.730	12			